Stoichiometric Network Analysis as Mathematical Method for Examinations of Instability Region and Oscillatory Dynamics

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Abstract: Reaction systems in chemistry, physical chemistry, and biochemistry, which can be described by true or pseudo-stoichiometric relationships between species, and, therefore, represented with stoichiometric models, are usually very complex. For the analysis of the models of these complex nonlinear reaction systems with more than three variables, which can be in different dynamic states like multistability, oscillatority or chaos, some general mathematical methods such as the Stoichiometric network analysis (SNA) must be used. Although the SNA is a powerful method for systematic examination of complex reaction systems, identification of underlying reaction pathways, and stability analysis of dynamic states, this method is practically unknown among mathematicians. Therefore, a simple application of SNA to one five-dimensional model is given here.

Keywords: Stoichiometric network analysis (SNA), mathematical modeling, stoichiometric models, nonlinear oscillatory reaction system, instability condition

1 Introduction

In complex nonlinear reaction system it is generally possible to find one or several regions in the parameter space where the main steady state is unstable. In these regions numerous self-organized dynamic states can be observed, such as multistability, oscillatority and chaos [12-14, 22, 27-29, 31, 32, 36, 38]. Although the mentioned dissipative structures are very common in nature (for example, almost all biochemical processes are in oscillatory dynamic states [12-15, 17, 22-24, 26-29, 31, 32, 36, 38]), the region of parameter space where they appear is often very narrow. Mathematical modeling of these complex processes is therefore

of essential importance, as it allows us to explore the parameter space systematically and identify the instability regions. If the investigated process is simple and the model can be reduced to two or three variables, the locus of unstable steady (nonequilibrium stationary) states can be easily obtained. For the analysis of models with more variables, some general methods must be used, such as the Stoichiometric Network Analysis (SNA) [3-7, 21]. The SNA is a powerful method for systematic examination of the models of complex reaction systems, identification of underlying reaction pathways, and stability analysis of dynamic states [3-12, 16-21, 23-26, 32-35]. However, this method is presented in literature in such a manner that it is not appropriately adapted to mathematicians, and, on the other side, also to chemists, physical chemist or biochemists, resulting in antagonism of scientist against this procedure [3-7]. With aim to exceed mentioned problem, we shall present here the SNA procedure applied to one five-dimensional model of a complex nonlinear reaction system that is derived from the model of the Bray-Liebhafsky oscillatory reaction [1, 2].

2 Model

Generally, in reaction systems, one or more initially present species, the reactants, give rise to one or more species that are not further transformed, the products, through a number of intermediate species that are produced and consumed during the course of the reaction. Hence, there are two fundamentally different types of species. The reactants and products are called external species. The intermediates that are produced and consumed during time-evolution of the process and do not appear in the net reaction are called internal species. The SNA studies the time evolution of the internal species for given concentrations of the external ones. In the proposed model Reactant R gives the product P *via* five intermediate species: $X_1,...,X_5$.

Reactions	no.
$X_3 \rightarrow X_4 + X_5$	(R1)
$X_4 + X_5 \rightarrow X_3$	(R2)
$X_3 + X_5 \rightarrow X_2$	(R3)
$X_2 \rightarrow 2X_4$	(R4)
$2X_4 \rightarrow X_2$	(R5)
$X_3 + X_4 \rightarrow X_1$	(R6)
$X_1 \rightarrow X_3 + X_4$	(R7)
$R + X_4 \rightarrow X_3 + P$	(R8)
$R + X_2 \rightarrow X_4 + X_5$	(R9)

Any reversible reaction can be written as two opposite forward reactions, in our case (R1) - (R2), (R4) - (R5) and (R6) - (R7). The correspondence with the chemical model [33] is given by $X_1 = I_2$, $X_2 = I_2O$, $X_3 = I^-$, $X_4 = HOI$ and $X_5 = HIO_2$.

Denoting the concentrations of the intermediates X_i ($i = 1, ..., 5$) by x_i, the time evolution of the system can be described by the following set of differential equations based on the proposed model and mass-action kinetics (see Appendix 1) [30, 37]:

$$\frac{dx_1}{dt} = k_6 x_3 x_4 - k_7 x_1 \tag{1}$$

$$\frac{dx_2}{dt} = k_3 x_3 x_5 - k_4 x_2 + k_5 x_4^2 - k_9 x_2 x_6 \tag{2}$$

$$\frac{dx_3}{dt} = -k_1 x_3 + k_2 x_4 x_5 - k_3 x_3 x_5 - k_6 x_3 x_4 + k_7 x_1 + k_8 x_4 x_6 \tag{3}$$

$$\frac{dx_4}{dt} = k_1 x_3 - k_2 x_4 x_5 + 2k_4 x_2 - 2k_5 x_4^2 - k_6 x_3 x_4 + k_7 x_1 - k_8 x_4 x_6 + k_9 x_2 x_6 \tag{4}$$

$$\frac{dx_5}{dt} = k_1 x_3 - k_2 x_4 x_5 - k_3 x_3 x_5 + k_9 x_2 x_6 \tag{5}$$

Here k_j ($j = 1, ..., 9$) denote the rate constants. From a mathematical point of view, in these equations, k_j are the parameters, whereas the concentrations x_i ($i = 1, ..., 5$) of intermediate species, are variables determining dynamic state of the considered reaction system. Therefore, the number of intermediate species defines the number of differential equations necessary to describe time evolution of the examined system in the concentration phase space, and, thus, determines the dimension of the analyzed problem.

The above differential equations can be written as a function of reaction rates v_j ($j = 1, ..., 9$) given by the expressions: $v_1 = k_1 x_3$, $v_2 = k_2 x_4 x_5$, $v_3 = k_3 x_3 x_5$, $v_4 = k_4 x_2$, $v_5 = k_5 x_4^2$, $v_6 = k_6 x_3 x_4$, $v_7 = k_7 x_1$, $v_8 = k_8 x_4 x_6$ and $v_9 = k_9 x_2 x_6$:

$$\frac{dx_1}{dt} = v_6 - v_7 \tag{6}$$

$$\frac{dx_2}{dt} = v_3 - v_4 + v_5 - v_9 \tag{7}$$

$$\frac{dx_3}{dt} = -v_1 + v_2 - v_3 - v_6 + v_7 + v_8 \tag{8}$$

$$\frac{dx_4}{dt} = v_1 - v_2 + 2v_4 - 2v_5 - v_6 + v_7 - v_8 + v_9 \tag{9}$$

$$\frac{dx_5}{dt} = v_1 - v_2 - v_3 + v_9 \tag{10}$$

Thus, we are dealing with a model given by nine stoichiometric relations between five species. The time evolution of their concentrations is described by corresponding system of

nonlinear first order differential equations (1.1-1.5). This system of differential equations is defined by law of mass action and, therefore, it is not arbitrary. Consequently, any modifications in modelling must be performed starting from the stoichiometric model. In the mathematical literature, the system of differential equations is often taken as the model and the main postulates as well as modifications in the modelling ought to be carried out on it.

The modeling of reaction systems by stoichiometric model has important advantage, since the basic model of reaction mechanism is universal for considered process. Therefore, when modification is necessary it can be easily updated, whereas the sophistically composed system of differential equations, in the other case, must be postulated from the beginning for every new demand. The SNA is the simple method for examination of such stoichiometric models of complex nonlinear processes, identification of underlying reaction pathways, and stability analysis of dynamic states.

3   Stoichiometric Network Analysis (SNA)

Modeling complex nonlinear processes requires a deep understanding of dynamical system theory, beside the standard kinetic examinations. In the case of complex nonlinear reaction systems, such as oscillatory reactions, the stability of the steady states needs to be examined in different regions of the parameter space.

As the concentrations of intermediate species are the dynamical variables, their number defines the number of differential equations needed to describe the dynamics of the examined system in the concentration phase space, and, thus, determines the dimensionality of this phase space. Consequently, a stability analysis will yield a *characteristic equation* whose order is equal to the number n of these intermediates. If a reaction system consists of two or three intermediate species ($n = 2$ or 3) the stability analysis can be performed simply by using the standard mathematical procedure [14] starting from the linearized kinetic equations in the vicinity of the steady states. The SNA also starts from the linearized kinetic equations in the vicinity of a steady state. However, it avoids direct solving of the n-dimensional characteristic equation of the Jacobian matrix which is practically unsolvable for $n > 3$.

The fundamental idea of SNA is to perform the stability analysis in the rates space instead of the concentrations space. It uses new parameters derived from the analysis of reaction routes or pathways of the considered process in the steady state. Any linear combination of the elementary steps that results in some net stoichiometric reaction without intermediate species therein is one reaction route or pathway. In terminology of SNA, the steady state (or nonequilibrium stationary state) reaction pathways are currents. Clarke [3-5] has shown that all the currents form a convex cone in the rate space, a 9 dimensional space in our example. The edges of this cone are called extreme currents. The first aim of SNA is to determine all extreme currents. For this, we first transform kinetic equations to a

matrix form and define the reaction pathways.

3.1 Matrix Form of Kinetic Equations

The differential equations (6)-(10) may be written in the matrix form (11).

$$\frac{dx_1}{dt} = 0v_1 + 0v_2 + 0v_3 + 0v_4 + 0v_5 + 1v_6 - 1v_7 + 0v_8 + 0v_9$$

$$\frac{dx_2}{dt} = 0v_1 + 0v_2 + 1v_3 - 1v_4 + 1v_5 + 0v_6 - 0v_7 + 0v_8 - 1v_9$$

$$\frac{dx_3}{dt} = -1v_1 + 1v_2 - 1v_3 + 0v_4 + 0v_5 - 1v_6 + 1v_7 + 1v_8 + 0v_9 \qquad (11)$$

$$\frac{dx_4}{dt} = 1v_1 - 1v_2 + 0v_3 + 2v_4 - 2v_5 - 1v_6 + 1v_7 - 1v_8 + 1v_9$$

$$\frac{dx_5}{dt} = 1v_1 - 1v_2 - 1v_3 + 0v_4 + 0v_5 + 0v_6 + 0v_7 + 0v_8 + 1v_9$$

The coefficients in these five equations form a matrix **S**, called stoichiometric matrix.

$$
\mathbf{S} =
\begin{array}{c}
\begin{array}{ccccccccc}
\text{R1} & \text{R2} & \text{R3} & \text{R4} & \text{R5} & \text{R6} & \text{R7} & \text{R8} & \text{R9}
\end{array} \\
\left[
\begin{array}{rrrrrrrrr}
0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 \\
0 & 0 & 1 & -1 & 1 & 0 & 0 & 0 & -1 \\
-1 & 1 & -1 & 0 & 0 & -1 & 1 & 1 & 0 \\
1 & -1 & 0 & 2 & -2 & -1 & 1 & -1 & 1 \\
1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 1
\end{array}
\right]
\begin{array}{l}
X_1 \\ X_2 \\ X_3 \\ X_4 \\ X_5
\end{array}
\end{array}
\qquad (12)
$$

Thus, the kinetic equations necessary for analysis of dynamic states of the Model (R1)-(R9) have the following matrix form:

$$
\begin{bmatrix}
\frac{dx_1}{dt} \\
\frac{dx_2}{dt} \\
\vdots \\
\frac{dx_5}{dt}
\end{bmatrix}
= S \cdot
\begin{bmatrix}
v_1 \\
v_2 \\
\vdots \\
v_9
\end{bmatrix}
\qquad (13)
$$

The corresponding stationary equation is (14) where v_{ss} is the one-column matrix of the reaction rates at the steady state.

$$\mathbf{S} \cdot v_{ss} = 0 \qquad (14)$$

In the general case, the concentrations of the intermediates in the steady states are functions of reactant concentrations, like R in our example. The SNA perform a stability analysis for given values of the reactant concentrations. In a steady state, the process as a whole

can be presented as a linear combination of several elementary reaction pathways or reaction routes with non-negative coefficients [3,5]. These elementary reaction pathways are the extreme currents E_i. They are obtained by identification of the right null vectors of the stoichiometric matrix. In proposed model it would give:

$$
\begin{bmatrix}
0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 \\
0 & 0 & 1 & -1 & 1 & 0 & 0 & 0 & -1 \\
-1 & 1 & -1 & 0 & 0 & -1 & 1 & 1 & 0 \\
1 & -1 & 0 & 2 & -2 & -1 & 1 & -1 & 1 \\
1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 1
\end{bmatrix}
\cdot
\begin{bmatrix}
e_{i,1} \\
e_{i,2} \\
\vdots \\
e_{i,9}
\end{bmatrix}
=
\begin{bmatrix}
0 \\
0 \\
\vdots \\
0
\end{bmatrix}
\tag{15}
$$

Since all elements of the vector on the right-hand side of equation (15) are equal to zero, the last expression is a homogeneous system of equations without unique solution. To avoid this problem, we use appropriate scaling relation of the reaction rates as additional equation: the sum of the e_i will be equal to one. Hence, a row with all elements equal to one is added in matrix S (Eq. (4)) before calculation of extreme currents, so Eq. (15) is transformed to Eq. (16) or Eq. (17) in matrix form.

$$
\begin{bmatrix}
0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 \\
0 & 0 & 1 & -1 & 1 & 0 & 0 & 0 & -1 \\
-1 & 1 & -1 & 0 & 0 & -1 & 1 & 1 & 0 \\
1 & -1 & 0 & 2 & -2 & -1 & 1 & -1 & 1 \\
1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 1 \\
1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1
\end{bmatrix}
\cdot
\begin{bmatrix}
e_{i,1} \\
e_{i,2} \\
\vdots \\
e_{i,9}
\end{bmatrix}_{ss}
=
\begin{bmatrix}
0 \\
0 \\
0 \\
0 \\
0 \\
1
\end{bmatrix}
\tag{16}
$$

$$
\mathbf{B} \cdot e_{ss} = b \tag{17}
$$

Rank of the corresponding stoichiometric matrix $\mathbf{S}$ is $n = 5$. The rank of matrix B is equal to $n + 1$. Therefore, there are $n + 1$ independent columns in this matrix. Any combination of $n + 1$ columns could give one independent reaction pathway in the steady state. However, some combinations lead to the same solution, others do not have solution at all and some combinations have solutions that could be decomposed to combinations of other ones. Nevertheless, all combinations of $n + 1$ columns will give all extreme currents. Selection of $n + 1$ from total number of m columns of the matrix S is equivalent to demand that other $m - (n + 1)$ e_i are zero for the considered reaction pathway. Finally, the e_i can be multiplied by common factor with aim to obtain small integer numbers.

Hence, all combinations of $n+1 = 6$ columns of B matrix will give all extreme currents. For example, first six columns have no solution whereas combination of the columns corresponding to v_1, v_3, v_4, v_6, v_8 and v_9 gives following system of equations:

$$
\begin{bmatrix}
0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 \\
0 & 0 & 1 & -1 & 1 & 0 & 0 & 0 & -1 \\
-1 & 1 & -1 & 0 & 0 & -1 & 1 & 1 & 0 \\
1 & -1 & 0 & 2 & -2 & -1 & 1 & -1 & 1 \\
1 & -1 & -1 & 0 & 0 & 0 & 0 & 0 & 1 \\
1 & 1 & 1 & 1 & 1 & 1 & 1 & 1 & 1
\end{bmatrix}
\cdot
\begin{bmatrix}
e_{i,1} \\
0 \\
e_{i,3} \\
e_{i,4} \\
0 \\
e_{i,6} \\
0 \\
e_{i,8} \\
e_{i,9}
\end{bmatrix}_{ss}
=
\begin{bmatrix}
0 \\
0 \\
0 \\
0 \\
0 \\
1
\end{bmatrix}
\tag{18}
$$

with solution:

$$
e_{3,ss} = e_{8,ss} = e_{9,ss} = \frac{1}{3} \quad and \quad e_{1,ss} = e_{6,ss} = 0
$$

equivalent to

$$
e_{3,ss} = e_{8,ss} = e_{9,ss} = 1 \quad and \quad e_{1,ss} = e_{6,ss} = 0
\tag{19}
$$

So, we have just obtained one reaction pathway in the steady state, that is, one extreme current of the stoichiometric network represented by the Model (R1)-(R9). Other combinations of columns of the matrix B give three additional independent extreme currents. All mutually different irreducible solutions obtained for extreme currents are columns of the new matrix $\mathbf{E}$, where the order of columns is arbitrary:

$$E = \begin{array}{c} \begin{array}{cccc} E_1 & E_2 & E_3 & E_4 \end{array} \\ \left[\begin{array}{cccc} 1 & 0 & 0 & 0 \\ 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 \\ 0 & 1 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \\ 0 & 0 & 0 & 1 \end{array} \right] \begin{array}{c} R1 \\ R2 \\ R3 \\ R4 \\ R5 \\ R6 \\ R7 \\ R8 \\ R9 \end{array} \end{array} \qquad (20)$$

Any column of the current matrix $\mathbf{E}$ represents one combination of reaction steps (the reactions of the model) that ensure net reaction without intermediates in it. In other words, in one reaction pathway any appearance of intermediates will be exactly compensated by their disappearance. In the considered example, the reactant and product are included only in the reactions (R8) and (R9) and, therefore, only in the fourth extreme current and also in the corresponding net reaction. Summing the reactions multiplied by the elements of E_4 we get:

$$X_3 + X_5 \rightarrow X_2$$
$$R + X_4 \rightarrow X_3 + P$$
$$R + X_2 \rightarrow X_4 + X_5$$
$$\Sigma: \quad 2R \quad \longrightarrow \quad P$$
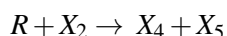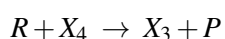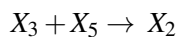
First three extreme currents are due to three reversible reactions, (R1) and (R2), (R4) and (R5), as well as (R6) and (R7), and therefore have no stoichiometric net result. For example E_1 gives

$$X_3 \rightarrow X_4 + X_5$$
$$X_4 + X_5 \rightarrow X_3$$
$$\Sigma: \quad 0 \quad \longrightarrow \quad 0$$
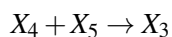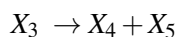
Thus, using SNA, we are able to analyze all reaction routes which is impossible to do without this method if the model is complex.

3.2   Current Rates

We have just seen that any simple reaction in a stationary state can give some contribution to the reaction pathways (extreme currents) where it takes place. Therefore, any reaction rate in the stationary state v_{ss} can be decomposed in current rates. The contributions of the extreme currents to the reactions rates, named *current rates* and denoted by j_i, are the components of the corresponding vector j. Thus, the basic equation of the stoichiometric network analysis is:

$$v_{ss} = \mathbf{E} \cdot j \tag{21}$$

Having the matrix $\mathbf{E}$, we can write the reaction rates at the steady state as linear combinations of the extreme current rates. These equations imply relations between the reaction rates at the steady state as well as the relations between the steady state concentrations. In the case of the Model (R1)-(R9) we obtain:

$$
\begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \\ v_6 \\ v_7 \\ v_8 \\ v_9 \end{bmatrix}_{ss}
=
\begin{bmatrix}
1 & 0 & 0 & 0 \\
1 & 0 & 0 & 0 \\
0 & 0 & 0 & 1 \\
0 & 1 & 0 & 0 \\
0 & 1 & 0 & 0 \\
0 & 0 & 1 & 0 \\
0 & 0 & 1 & 0 \\
0 & 0 & 0 & 1 \\
0 & 0 & 0 & 1
\end{bmatrix}
\cdot
\begin{bmatrix} j_1 \\ j_2 \\ j_3 \\ j_4 \end{bmatrix}
\tag{22}
$$

that is:

$$
\begin{aligned}
v_{1,ss} &= v_{2,ss} = j_1 \\
v_{3,ss} &= it\, v_{8,ss} = v_{9,ss} = j_4 \\
v_{4,ss} &= v_{5,ss} = j_2 \\
v_{6,ss} &= v_{7,ss} = j_3
\end{aligned}
\tag{23}
$$

Let us underline that, in the general case, reaction rates are linear combinations of current rates. Taking into account rates of reactions (R1)-(R9) based on mass action law, given under Eqs. (6)-(10) and (23), steady state reaction rates can be expressed by means of both classic kinetic (rate constants and steady state concentrations) or SNA (current rates) parameters:

$$v_{1,ss} = v_{2,ss} = k_1 x_{3,ss} = k_2 x_{4,ss} x_{5,ss} = j_1$$
$$v_{3,ss} = v_{8,ss} = v_{9,ss} = k_3 x_{3,ss} x_{5,ss} = k_8 x_{4,ss} x_{6,0} = k_9 x_{2,ss} x_{6,0} = j_4$$
$$v_{4,ss} = v_{5,ss} = k_4 x_{2,ss} = k_5 x_{4,ss}^2 = j_2 \tag{24}$$
$$v_{6,ss} = v_{7,ss} = k_6 x_{3,ss} x_{4,ss} = k_7 x_{1,ss} = j_3$$

Here there are five steady state concentrations of intermediary species X_i ($i = 1, ..., 5$) and the concentration of reactant X_6. Since the concentration of reactant X_6 is much higher than the concentration of other species, and therefore x_6 changes much more slowly than the concentration of the intermediate species, it is taken as constant in SNA with value $x_{6,0}$ in further calculations. The relations (24) are particularly useful for evaluation of the steady state concentrations. Thus, from five relations between five stationary state concentrations given in equations (24), we can easily obtain their values. They are

$$x_{1,ss} = \frac{k_6}{k_7} \frac{k_2}{k_1} \left(\frac{k_8 k_4}{k_9 k_5} \right)^2 \sqrt{\frac{k_8 k_1}{k_3 k_2} x_{6,0}}$$
$$x_{2,ss} = \frac{k_5}{k_4} \left(\frac{k_8 k_4}{k_9 k_5} \right)^2$$
$$x_{3,ss} = \frac{k_2}{k_1} \frac{k_8 k_4}{k_9 k_5} \sqrt{\frac{k_8 k_1}{k_3 k_2} x_{6,0}} \tag{25}$$
$$x_{4,ss} = \frac{k_8 k_4}{k_9 k_5}$$
$$x_{5,ss} = \sqrt{\frac{k_8 k_1}{k_3 k_2} x_{6,0}}$$

The advantage of this procedure where we calculate steady state concentrations using the relations between reaction rates and current rates can be seen better on larger models, where traditional calculations are practically impossible.

3.3   The Stability Analysis

The next step in SNA is to investigate the stability of the network's motion around a steady state after an infinitely small perturbation, that is, to find the instability condition and the region of parameters where the steady state of the model is unstable. The dynamics of small concentration perturbations $\Delta x = x - x_{ss}$ near a steady state x_{ss} is given by the equation

$$\frac{d\Delta x}{dt} = M \Delta x. \tag{26}$$

obtained by linearization of the general equation of motion about this steady state. Its stability depends on the sign of the real part of the eigenvalues of the Jacobian matrix $\mathbf{M}$ given in the form

$$\mathbf{M} = S \, (\text{diag } v_{\text{ss}}) \, \mathbf{K}^{\text{T}} \, (\text{diag } x_{\text{ss}}^{-1}). \tag{27}$$

Here, $\mathbf{K}$ is the matrix of the orders of reaction and $\mathbf{K}^{T}$ is its transpose. In the SNA [3] the matrix $\mathbf{M}$ is written as

$$\mathbf{M} = S \, (\text{diag } \mathbf{E}j) \, \mathbf{K}^{\text{T}} \, (\text{diag } h), \tag{28}$$

where diag $\boldsymbol{h}$ is a diagonal matrix whose elements are the reciprocals of steady state concentrations ($h_i = 1/x_{i,\text{ss}}$) and diag $\mathbf{E}j$ is a diagonal matrix whose elements are the reaction rates at the steady state. The matrix $\mathbf{M}$ written as function of the SNA parameters has particular advantages for the stability analysis since the parameters j_i and h_i are non-negative, what is an essential feature of the SNA. The steady state stability is determined by the eigenvalues of $\mathbf{M}$, which are the roots λ of the characteristic polynomial

$$Det \, [\lambda \, \mathbf{I} - \mathbf{M}] = \lambda^{5} + \lambda^{4} \alpha_1 + \lambda^{3} \alpha_2 + \lambda^{2} \alpha_3 + \lambda \, \alpha_4 + \alpha_5 = 0. \tag{29}$$

where α_i ($i = 1,2,\ldots,5$) is the coefficient of the corresponding eigenvalue λ^{5-i}, and $\mathbf{I}$ is the unit matrix. By convention, $\alpha_0 = 1$. Since diag $\boldsymbol{h}$ introduces only scaling factors, it is useful to define a *current rate matrix* $\mathbf{V}(j)$, given by the expression:

$$\mathbf{V}(j) = - S \, \text{diag} \, (Ej) \, K^{\text{T}}. \tag{30}$$

so that $\mathbf{M} = -\mathbf{V}(j) \, (\text{diag } h)$. As any α_i is the sum of minors of $\mathbf{V}(j)$ with dimension i multiplied by the product of the corresponding h_i values, we look for a negative diagonal minors of $\mathbf{V}(j)$. If we find negative minors in one α_i we ask if the sum of negative terms can be larger than the sum of positive terms in same α_i. In the model considered here, several minors can be negative so that the steady state can be unstable.

If the underlying kinetics occurs according to the law of mass action, $\mathbf{K}^{T}$ in the last equation represents the transpose of the *matrix of reaction orders*, whose elements are general stoichiometric coefficients of species X_i standing on the left side of the reaction j, $s_{i,j}^{\text{L}}$ (see Eq. (A1.1), Appendix 1). In the considered case, the matrix $\mathbf{K}$ is

$$\mathbf{K} = \begin{bmatrix} 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 1 \\ 1 & 0 & 1 & 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 2 & 1 & 0 & 1 & 0 \\ 0 & 1 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \end{bmatrix} \quad (31)$$

By substituting matrixes $\mathbf{K}$, $\mathbf{S}$, and $\mathbf{E}$ in equation (30), the matrix of currents rates $\mathbf{V}(j)$ can be derived:

$$\mathbf{V}(j) = \begin{bmatrix} j_3 & 0 & -j_3 & -j_3 & 0 \\ 0 & j_2 + j_4 & -j_4 & -2j_2 & -j_4 \\ -j_3 & 0 & j_1 + j_3 + j_4 & -j_1 + j_3 - j_4 & -j_1 + j_4 \\ -j_3 & -2j_2 - j_4 & -j_1 + j_3 & j_1 + 4j_2 + j_3 + j_4 & j_1 \\ 0 & -j_4 & -j_1 + j_4 & j_1 & j_1 + j_4 \end{bmatrix} \quad (32)$$

Steady state is unstable if and only if one root of the characteristic equation Eq. (29) has a positive real part and the general instability condition is given by the Routh-Hurwitz criteria. However, even with the simplifications of the SNA equations, these criteria are unusable for large models. In practice, it is found that we can use a much simpler criterion: we look for conditions where a coefficient a_i can be negative (a approximation) [6, 21]. Moreover, the parameters j_i and h_i are non-negative so that any negative term is preceded by a minus sign that can be easily located in complicated equations using symbolic calculations.

Coefficients α_i of the characteristic equation Eq. (29) represent the sum of all diagonal minors M of dimensions $i \times i$, ($i = 1, \ldots, 5$) of the matrix $\mathbf{V}(j)$ multiplied by corresponding sets of reciprocal concentrations h_i [12, 18, 20]. Thus, coefficients α_1, α_2 and α_5 are:

$$\alpha_1 = j_3 h_1 + (j_2 + j_4) h_2 + (j_1 + j_3 + j_4) h_3 + (j_1 + 4j_2 + j_3 + j_4) h_4 + (j_1 + j_4) h_5 = Tr(\mathbf{M}) \quad (33)$$

$$\alpha_2 = j_3\,(j_2+j_4)\,h_1h_2 + \left[j_3\,(j_1+j_3+j_4)-j_3^2\right]h_1h_3 + \left[j_3\,(j_1+4j_2+j_3+j_4)-j_3^2\right]h_1h_4 +$$
$$+ j_3\,(j_1+j_4)\,h_1h_5 + (j_2+j_4)\,(j_1+j_3+j_4)\,h_2h_3 +$$
$$+ \left[(j_2+j_4)\,(j_1+4j_2+j_3+j_4)-2j_2\,(2j_2+j_4)\right]h_2h_4 +$$
$$+ \left[(j_2+j_4)\,(j_1+j_4)-j_4^2\right]h_2h_5 +$$
$$+ \left[(j_1+j_3+j_4)\,(j_1+4j_2+j_3+j_4)-(-j_1+j_3-j_4)\,(-j_1+j_3)\right]h_3h_4 +$$
$$+ \left[(j_1+j_3+j_4)\,(j_1+j_4)-(-j_1+j_4)^2\right]h_3h_5 +$$
$$+ \left[(j_1+4j_2+j_3+j_4)\,(j_1+j_4)-j_1^2\right]h_4h_5 = \qquad (34)$$
$$= j_3\,(j_2+j_4)\,h_1h_2 + j_3\,(j_1+j_4)\,h_1h_3 + j_3\,(j_1+4j_2+j_4)\,h_1h_4 +$$
$$+ j_3\,(j_1+j_4)\,h_1h_5 + (j_2+j_4)\,(j_1+j_3+j_4)\,h_2h_3 +$$
$$+ \left[j_2\,(j_1+j_3+3j_4)+j_4\,(j_1+j_3+j_4)\right]h_2h_4 +$$
$$+ \left[(j_2+j_4)\,j_1+j_2j_4\right]h_2h_5 + \left[4\,(j_1j_2+j_1j_3+j_2j_3)+j_4\,(j_1+4j_2+3j_3+j_4)\right]h_3h_4 +$$
$$+ \left[4j_1j_4+j_3\,(j_1+j_4)\right]h_3h_5 + \left[j_1\,(4j_2+j_3+j_4)+j_4\,(j_1+4j_2+j_3+j_4)\right]h_4h_5$$

$$\alpha_5 = 2j_1j_2j_3j_4^2\,h_1h_2h_3h_4h_5 \qquad (35)$$

Obviously, the coefficients α_1, α_2 and α_5 are always positive and therefore the source of instability is not in them. However, α_3 and α_4 may be negative. Instead to calculate all α_i, we shall look for negative minors in matrix $\mathbf{V}(j)$. There are three possibly negative minors:

$$M_{245} = 2j_1j_4^2 - j_2j_4^2 + j_1j_2j_3 + 8j_1j_2j_4 + j_1j_3j_4 + j_2j_3j_4. \qquad (36)$$
$$M_{1245} = 2j_1j_3j_4^2 - j_2j_3j_4^2 + 8j_1j_2j_3j_4. \qquad (37)$$
$$M_{2345} = 2j_1j_2j_4^2 - 2j_2j_3j_4^2 + 8j_1j_3j_4^2 + 23j_1j_2j_3j_4. \qquad (38)$$

Thus, only α_3 and α_4 can be negative. When negative term is dominant, corresponding α_i is negative, and there exists positive real part of some root of the characteristic equation. Under these conditions, the examined steady state is unstable. Since minors M_{1245} and M_{2345}, both include minor M_{245}, the source of instability is in the last one. The instability condition which results from minor M_{245} is:

$$j_2j_4^2 > 2j_1j_4^2 + j_1j_2j_3 + 8j_1j_2j_4 + j_1j_3j_4 + j_2j_3j_4. \qquad (39)$$

Besides, term $M_{245}h_2h_4h_5$ must be dominant if we want to have negative α_3. Since, h_i being reciprocal concentrations, this term is dominant if the concentrations of the intermediate species X_2, X_4 and X_5 are sufficiently low. This is true for the considered chemical system so that the instability is approximately defined by the simplified instability condition (39). The minor M_{245} is the core of the instability of the model.

By means of relations (23), the instability condition (39) can be rewritten in function of the steady state reaction rates. There are several combinations of the reaction rates that satisfy mentioned equations. We can select any of them, but we always prefer the one for which we have the best experimental evidence. One possible expression for the instability condition is:

$$v_5 v_3^2 > 2v_1 v_3^2 + v_1 v_5 v_7 + 8v_1 v_5 v_3 + v_1 v_7 v_3 + v_5 v_7 v_3. \tag{40}$$

The subscript "ss" is dropped in the last expression for simplicity. The equation (40) can be also written in dimensionless form. Such kind of discussion is important for practical purposes and, although it is not necessary for further calculations here, the dimensionless kinetic equations are given in Appendix 2.

Thus, since any polynomial of related minor can be expressed as function of steady state reaction rates, by SNA we can evaluate the instability condition as function of them and correlate it with experimentally obtained values for corresponding rate constants and steady state concentrations. Moreover, as the steady state concentrations are also functions of rate constants, we can express the instability condition as function of rate constants and initial concentrations of reactants, only. Calculated instability is located in a defined domain of the phase space where we can analyze dynamic states by numerical simulations. Without mentioned calculations we had a serious problem to find the instability region, since the parameters, rate constants, differs from one another for several (frequently more than ten) order of magnitudes.

Moreover, the SNA allows the distinction between different kinds of instability. Taking into account the above calculated α_i we can construct all Hurwitz determinants $?_i$, $i = 1,\ldots,n$, which are defined as the determinants of the leading principal minor, made from the first i rows and columns of the Hurwitz matrix $\mathbf{H}$, where $a_i = 0$ for $i > n$ (in the analyzed model $n = 5$).

$$\mathbf{H} = \begin{bmatrix} \alpha_1 & \alpha_3 & \alpha_5 & \alpha_7 & \ldots & \alpha_{2n-1} \\ 1 & \alpha_2 & \alpha_4 & \alpha_6 & \ldots & \alpha_{2n-2} \\ 0 & \alpha_1 & \alpha_3 & \alpha_5 & \ldots & \alpha_{2n-3} \\ 0 & 1 & \alpha_2 & \alpha_4 & \ldots & \alpha_{2n-4} \\ 0 & 0 & \alpha_1 & \alpha_3 & \ldots & \alpha_{2n-5} \\ \vdots & \vdots & \vdots & \vdots & \ddots & \vdots \\ 0 & 0 & 0 & 0 & \ldots & \alpha_n \end{bmatrix}. \tag{41}$$

A saddle-node bifurcation occurs when the condition $\alpha_n = 0$ is satisfied [24]. For the considered model,

$$\alpha_n = \alpha_5 = 2j_1j_2j_3j_4^2 h_1h_2h_3h_4h_5 > 0 \tag{42}$$

Thus, a saddle-node bifurcation doesn't occur. An Andronov-Hopf bifurcation occurs when $\Delta_{n-1} = 0$ [7, 24]. For the considered model,

$$\Delta_4 = \det \begin{bmatrix} \alpha_1 & \alpha_3 & \alpha_5 & 0 \\ 1 & \alpha_2 & \alpha_4 & 0 \\ 0 & \alpha_1 & \alpha_3 & \alpha_5 \\ 0 & 1 & \alpha_2 & \alpha_4 \end{bmatrix} = 0 \tag{43}$$

or

$$\Delta_4 = \left(\alpha_1\alpha_2\alpha_3\alpha_4 + 2\alpha_1\alpha_4\alpha_5 + \alpha_2\alpha_3\alpha_5\right) - \left(\alpha_1\alpha_2^2\alpha_5 + \alpha_1^2\alpha_4^2 + \alpha_3^2\alpha_4 + \alpha_5^2\right) = 0 \tag{44}$$

Both, α_3 and α_4 can be negative, probably simultaneously, while the other coefficients are always positive. Grouping the positive terms on the left and the potentially negative ones on the right side of equation, the condition for the Andronov-Hopf bifurcation becomes:

$$\alpha_1\alpha_2\alpha_3\alpha_4 - \alpha_3^2\alpha_4 = \left(\alpha_1\alpha_2^2\alpha_5 + \alpha_1^2\alpha_4^2 + \alpha_5^2\right) - \left(2\alpha_1\alpha_4\alpha_5 + \alpha_2\alpha_3\alpha_5\right) \tag{45}$$
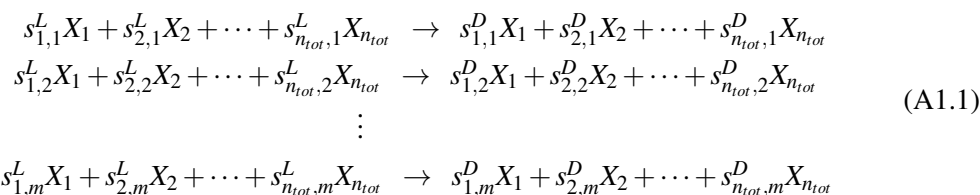
Minor M_{245} remains the most probable source of the instability, and hence, the instability condition (39) or (40) can be used for the approximate identification of the Andronov-Hopf bifurcation.

4   Conclusion

The stoichiometric network analysis is applied to a five-dimensional model of a complex nonlinear reaction system with aim to attract readers to use this method in any other mathematical modeling of reaction systems that can be presented by stoichiometric relations between species. In particular, the analytical procedure for calculations of reaction pathways, instability condition and dimensionless equations, all in a model with five variables, was explicitly shown. Practical SNA-based approach is also given, to differentiate between saddle node and Andronov-Hopf bifurcations leading to instability and oscillations.

Appendix 1: Differential equations based on the mass-action kinetics, matrix form of kinetic equations and stationary states

Any reaction system consisted of m reactions and n_{tot} species denoted by X_i ($i = 1, ..., n_{\text{tot}}$), where n_{tot} is the total number of species including both internal (n) and external (n_{ex}) ones, can be presented in the following form:

$$s_{1,1}^L X_1 + s_{2,1}^L X_2 + \cdots + s_{n_{tot},1}^L X_{n_{tot}} \quad \rightarrow \quad s_{1,1}^D X_1 + s_{2,1}^D X_2 + \cdots + s_{n_{tot},1}^D X_{n_{tot}}$$
$$s_{1,2}^L X_1 + s_{2,2}^L X_2 + \cdots + s_{n_{tot},2}^L X_{n_{tot}} \quad \rightarrow \quad s_{1,2}^D X_1 + s_{2,2}^D X_2 + \cdots + s_{n_{tot},2}^D X_{n_{tot}}$$
$$\vdots$$
$$s_{1,m}^L X_1 + s_{2,m}^L X_2 + \cdots + s_{n_{tot},m}^L X_{n_{tot}} \quad \rightarrow \quad s_{1,m}^D X_1 + s_{2,m}^D X_2 + \cdots + s_{n_{tot},m}^D X_{n_{tot}}$$

$$(A1.1)$$

Here, $s_{i,j}^L$ and $s_{i,j}^D$ are the stoichiometric coefficients of considered species at left (L) and right (D) side of equation. Index j denotes the ordinal number of a reaction in a series of m reactions. The stoichiometric coefficients are usually small integer numbers ($s_{i,j} = 0, 1, 2, ...$). If stoichiometric coefficient is zero at one or both sides of equation, this species does not appear in stoichiometric expression at this or both sides, respectively. Besides, one species can appear on both sides of chemical reaction, as it is the case in direct autocatalytic or autoinhibitory reactions. All reactions can be either simple forward or reversible ones. Any reversible reaction can be written as two forward ones. In SNA, the reverse reactions must be taken as two forward ones. Therefore we shall consider the system of forward reactions only.

Denoting by x_i the concentration of species X_i, and by $S_{i,j} = s_{i,j}^D - s_{i,j}^L$ the difference between its stoichiometric coefficients from the right and left side of reaction j, we can write the kinetic equations that correspond to the model (A1.1) in the following form:

$$\frac{dx_1}{dt} = S_{1,1}v_1 + S_{1,2}v_2 + \cdots + S_{1,m}v_m$$
$$\frac{dx_2}{dt} = S_{2,1}v_1 + S_{2,2}v_2 + \cdots + S_{2,m}v_m$$
$$\vdots$$
$$\frac{dx_{n_{tot}}}{dt} = S_{n_{tot},1}v_1 + S_{n_{tot},2}v_2 + \cdots + S_{n_{tot},m}v_m$$

$$(A1.2)$$

Here the reaction rates v_j are functions of rate constants k_j and concentrations of the species that take place in reaction step j. They are usually expressed as the product of the corresponding rate constant and relevant species concentrations with respective exponents equal to stoichiometric coefficient:

$$v_j = k_j x_1^{s_{1,j}^L} x_2^{s_{2,j}^L} \cdots x_{n_{tot}}^{s_{n_{tot},j}^L} \qquad (A1.3)$$

Let us consider reaction rates of three reactions:

Reactions		no.
$X_2 \rightarrow 2X_4$	$v_4 = k_4 x_2$	(R4)
$2X_4 \rightarrow X_2$	$v_5 = k_5 x_4^2$	(R5)
$X_2 + 2X_4 \rightarrow 3X_4$	$v_{10} = k_{10} x_2 x_4^2$	(R10)

If we imagine above three reactions as the model of a process, the time evolution of the particular species X_2 and X_4 is described by the following differential equations:

$$\frac{dx_2}{dt} = -k_4 x_2 + k_5 x_4^2 - k_{10} x_2 x_4^2$$
$$\frac{dx_4}{dt} = 2k_4 x_2 - 2k_5 x_4^2 + k_{10} x_2 x_4^2$$

(A1.4)

The sign + in the second equation before the rate of reaction (R10) is a consequence of the fact that in this reaction there are three molecules of species X_4 on the right side and two of them on the left side. That is, the concentration of this species in considered reaction will increase.

In the general case, the kinetic Eq. (A1.2) can be simply written in the matrix form, by the operator named the *stoichiometric matrix* S_{tot}:

$$\mathbf{S}_{tot} = \begin{array}{c} \begin{array}{cccc} R_1 & R_2 & \cdots & R_m \end{array} \\ \left[\begin{array}{cccc} S_{1,1} & S_{1,2} & \cdots & S_{1,m} \\ S_{2,1} & S_{2,2} & \cdots & S_{2,m} \\ \vdots & \vdots & \ddots & \vdots \\ S_{n_{tot},1} & S_{n_{tot},2} & \cdots & S_{n_{tot},m} \end{array} \right] \begin{array}{c} X_1 \\ X_2 \\ \vdots \\ X_{n_{tot}} \end{array} \end{array}$$

(A1.5)

In the literature, the elements $S_{i,j} = s_{i,j}^D - s_{i,j}^L$ are usually called stoichiometric coefficients, although there is obvious difference between $S_{i,j}$ and stoichiometric coefficients $s_{i,j}^D$ and $s_{i,j}^L$.

In Eq. (A1.5), the row over the matrix S_{tot} denotes reactions, whereas the column on the right side denotes reaction species. This extra row and column are not part of the matrix S_{tot}, but help a reader to correlate their elements with corresponding reactions and species, selected in arbitrary order.

Since, in stoichiometric network analysis, the reverse reactions must be taken as two forward ones, every reverse reaction has two columns in stoichiometric matrix S_{tot}. The system of kinetic equations in the matrix form is:

$$\begin{bmatrix} \frac{dx_1}{dt} \\ \frac{dx_2}{dt} \\ \vdots \\ \frac{dx_{n_{tot}}}{dt} \end{bmatrix} = \begin{bmatrix} S_{1,1} & S_{1,2} & \cdots & S_{1,m} \\ S_{2,1} & S_{2,2} & \cdots & S_{2,m} \\ \vdots & \vdots & \ddots & \vdots \\ S_{n_{tot},1} & S_{n_{tot},2} & \cdots & S_{n_{tot},m} \end{bmatrix} \cdot \begin{bmatrix} v_1 \\ v_2 \\ \vdots \\ v_m \end{bmatrix}$$

(A1.6)

The same equation can also be written in the form:

$$\frac{dx}{dt} = \mathbf{S}_{tot} \cdot v$$

(A1.7)

where dx/dt is vector, that is, one-column reaction rate matrix.

In the *equilibrium stationary state*, or, simply, in the *equilibrium*, for any reaction species X_i, including reactants and products, the following equation must be satisfied:

$$\left(\frac{dx_i}{dt} \right)_{ss} = S_{i,1} v_{1,ss} + S_{i,2} v_{2,ss} + \cdots + S_{i,m} v_{m,ss} = 0$$

(A1.8)

Consequently, the equilibrium stationary state can be calculated from the following matrix relation:

$$S_{tot} \cdot v_{ss} = 0 \qquad (A1.9)$$

In the *nonequilibrium stationary state*, same equation must be satisfied, but only for independent intermediates X_i. If $(dx_i/dt)_{ss}$ for all of them are equal to zero, we shall obtain the main steady state. However, we can discuss also the steady states with respect to subsystems of independent intermediate species.

Thus, for the system of n independent intermediate species, the nonequilibrium stationary state is defined by the following matrix equation:

$$
\begin{bmatrix}
\frac{dx_1}{dt} \\
\frac{dx_2}{dt} \\
\vdots \\
\frac{dx_n}{dt}
\end{bmatrix}_{ss}
=
\begin{bmatrix}
S_{1,1} & S_{1,2} & \cdots & S_{1,m} \\
S_{2,1} & S_{2,2} & \cdots & S_{2,m} \\
\vdots & \vdots & \ddots & \vdots \\
S_{n,1} & S_{n,2} & \cdots & S_{n,m}
\end{bmatrix}
\cdot
\begin{bmatrix}
v_1 \\
v_2 \\
\vdots \\
v_m
\end{bmatrix}_{ss}
=
\begin{bmatrix}
0 \\
0 \\
\vdots \\
0
\end{bmatrix}
\qquad (A1.10)
$$

or in the following matrix form:

$$S \cdot v_{ss} = 0 \qquad (A1.11)$$

Thus, equation (A1.9) is related to *equilibrium*, while equation (A1.11) is related to *the non-equilibrium stationary states*, that is, *the steady states*. As we have already said, the equilibrium stationary states can be only stable and therefore uninteresting for further examinations of dynamic states of a system.

Appendix 2: Dimensionless kinetic equations

The dimensionless kinetic equations can be very useful. Among others, they can be used to reduce the number of parameters. The procedure for obtaining the dimensionless kinetic equations will be again demonstrated using the Model (R1)-(R9) as an example. There, we first need to define new dimensionless variables denoted for internal by β_i where $\beta_i = x_i/x_{i,ss}$ ($i = 1, ..., 5$), for reactant by $\beta_6 = x_6/x_{6,0}$ and for product by $\beta_7 = x_7/x_{6,0}$. Introducing them into the kinetic equations (6)-(10) we obtain:

$$
\begin{aligned}
x_{1,ss}\frac{d\beta_1}{dt} &= v_6 - v_7 \\
x_{2,ss}\frac{d\beta_2}{dt} &= v_3 - v_4 + v_5 - v_9 \\
x_{3,ss}\frac{d\beta_3}{dt} &= -v_1 + v_2 - v_3 - v_6 + v_7 + v_8 \\
x_{4,ss}\frac{d\beta_4}{dt} &= v_1 - v_2 + 2v_4 - 2v_5 - v_6 + v_7 - v_8 + v_9 \qquad (A2.1)\\
x_{5,ss}\frac{d\beta_5}{dt} &= v_1 - v_2 - v_3 + v_9 \\
x_{6,0}\frac{d\beta_6}{dt} &= -v_8 - v_9 \\
x_{6,0}\frac{d\beta_7}{dt} &= v_8
\end{aligned}
$$

The reaction rates presented here can be expressed in function of dimensionless concentrations, too, and the current rates in the steady state (24):

$$v_1 = k_1 x_{3,ss} \frac{x_3}{x_{3,ss}} = v_{1,ss}\beta_3 = j_1\beta_3$$

$$v_2 = k_2 x_{4,ss}x_{5,ss} \frac{x_4}{x_{4,ss}}\frac{x_5}{x_{5,ss}} = v_{2,ss}\beta_4\beta_5 = j_1\beta_4\beta_5$$

$$v_3 = k_3 x_{3,ss}x_{5,ss} \frac{x_3}{x_{3,ss}}\frac{x_5}{x_{5,ss}} = v_{3,ss}\beta_3\beta_5 = j_4\beta_3\beta_5$$

$$v_4 = k_4 x_{2,ss} \frac{x_2}{x_{2,ss}} = v_{4,ss}\beta_2 = j_2\beta_2$$

$$v_5 = k_5 x_{4,ss}^2 \left(\frac{x_4}{x_{4,ss}}\right)^2 = v_{5,ss}\beta_4^2 = j_2\beta_4^2 \qquad (A2.2)$$

$$v_6 = k_6 x_{3,ss}x_{4,ss} \frac{x_3}{x_{3,ss}}\frac{x_4}{x_{4,ss}} = v_{6,ss}\beta_3\beta_4 = j_3\beta_3\beta_4$$

$$v_7 = k_7 x_{1,ss} \frac{x_1}{x_{1,ss}} = v_{7,ss}\beta_1 = j_3\beta_1$$

$$v_8 = k_8 x_{4,ss}x_{6,0} \frac{x_4}{x_{4,ss}}\frac{x_6}{x_{6,0}} = v_{8,ss}\beta_4\beta_6 = j_4\beta_4\beta_6$$

$$v_9 = k_9 x_{2,ss}x_{6,0} \frac{x_2}{x_{2,ss}}\frac{x_6}{x_{6,0}} = v_{9,ss}\beta_2\beta_6 = j_4\beta_2\beta_6$$

The obtained reaction rates in the steady state can be introduced in the kinetic equation (A2.1). Further, dividing both sides of every equation with concentrations of the species that appears on the left-hand side, we shall obtain their reciprocal concentration values on the right-hand side. As already mentioned, they are new parameters in SNA, denoted by h_i. In the case of the considered model they are:

$$h_i = \frac{1}{x_{i,ss}}, \text{ where } i = 1,...,5, \text{ and } h_6 = \frac{1}{x_{6,0}}. \qquad (A2.3)$$

Taking into account relations (A2.2) and (A2.3) the kinetic equations (A2.1) can be written as function of the SNA parameters, but they are not yet in the dimensionless form. With this aim we shall introduce the dimensionless time by means of the rate constant of any first order reaction, for example reaction (R7):

$$d\tau = dt \cdot k_7 = dt \cdot k_7 x_{1,ss} \frac{1}{x_{1,ss}} = dt \cdot v_{7,ss} h_1 = dt \cdot j_3 h_1. \qquad (A2.4)$$

By substitution of all relations (A2.2), (A2.3) and (A2.4) into the Eq. (A2.1), we can simply obtain the dimensionless kinetic equations in the following form:

$$\frac{d\beta_1}{d\tau} = \beta_3\beta_4 - \beta_1$$

$$\frac{d\beta_2}{d\tau} = \frac{h_2}{h_1}\left(\frac{j_4}{j_3}\beta_3\beta_5 - \frac{j_2}{j_3}\beta_2 + \frac{j_2}{j_3}\beta_4^2 - \frac{j_4}{j_3}\beta_2\beta_6\right)$$

$$\frac{d\beta_3}{d\tau} = \frac{h_3}{h_1}\left(-\frac{j_1}{j_3}\beta_3 + \frac{j_1}{j_3}\beta_4\beta_5 - \frac{j_4}{j_3}\beta_3\beta_5 - \beta_3\beta_4 + \beta_1 + \frac{j_4}{j_3}\beta_4\beta_6\right)$$

$$\frac{d\beta_4}{d\tau} = \frac{h_4}{h_1}\left(\frac{j_1}{j_3}\beta_3 - \frac{j_1}{j_3}\beta_4\beta_5 + 2\frac{j_2}{j_3}\beta_2 - 2\frac{j_2}{j_3}\beta_4^2 - \beta_3\beta_4 + \beta_1 - \frac{j_4}{j_3}\beta_4\beta_6 + \frac{j_4}{j_3}\beta_2\beta_6\right) \qquad (A2.5)$$

$$\frac{d\beta_5}{d\tau} = \frac{h_5}{h_1}\left(\frac{j_1}{j_3}\beta_3 - \frac{j_1}{j_3}\beta_4\beta_5 - \frac{j_4}{j_3}\beta_3\beta_5 + \frac{j_4}{j_3}\beta_2\beta_6\right)$$

$$\frac{d\beta_6}{d\tau} = \frac{h_6}{h_1}\left(-\frac{j_4}{j_3}\beta_4\beta_6 - \frac{j_4}{j_3}\beta_2\beta_6\right)$$

$$\frac{d\beta_7}{d\tau} = \frac{h_6}{h_1}\frac{j_4}{j_3}\beta_4\beta_6$$

These equations can be additionally simplified by introduction of new dimensionless

parameters for any combination of current rates that appear several times. In the considered case they are:

$$\kappa_1 = \frac{j_1}{j_3}, \ \kappa_2 = \frac{j_2}{j_3} \ \text{and} \ \kappa_3 = \frac{j_4}{j_3}. \tag{A2.6}$$

Hence, the dimensionless equations can be transformed to the form:

$$\frac{d\beta_1}{d\tau} = \beta_3\beta_4 - \beta_1$$

$$\frac{d\beta_2}{d\tau} = \frac{h_2}{h_1}\left(\kappa_3\beta_3\beta_5 - \kappa_2\beta_2 + \kappa_2\beta_4^2 - \kappa_3\beta_2\beta_6\right)$$

$$\frac{d\beta_3}{d\tau} = \frac{h_3}{h_1}\left(-\kappa_1\beta_3 + \kappa_1\beta_4\beta_5 - \kappa_3\beta_3\beta_5 - \beta_3\beta_4 + \beta_1 + \kappa_3\beta_4\beta_6\right)$$

$$\frac{d\beta_4}{d\tau} = \frac{h_4}{h_1}\left(\kappa_1\beta_3 - \kappa_1\beta_4\beta_5 + 2\kappa_2\beta_2 - 2\kappa_2\beta_4^2 - \beta_3\beta_4 + \beta_1 - \kappa_3\beta_4\beta_6 + \kappa_3\beta_2\beta_6\right) \tag{A2.7}$$

$$\frac{d\beta_5}{d\tau} = \frac{h_5}{h_1}\left(\kappa_1\beta_3 - \kappa_1\beta_4\beta_5 - \kappa_3\beta_3\beta_5 + \kappa_3\beta_2\beta_6\right)$$

$$\frac{d\beta_6}{d\tau} = \frac{h_6}{h_1}\left(-\kappa_3\beta_4\beta_6 - \kappa_3\beta_2\beta_6\right)$$

$$\frac{d\beta_7}{d\tau} = \frac{h_6}{h_1}\kappa_3\beta_4\beta_6$$

The dimensionless kinetic equations immediately show if the considered variable is slow or fast. Thus, in the considered case, concentration of the intermediate species X_1 has much higher steady state value than X_2, and hence, the scaling factor h_1 in the corresponding kinetic equation is much lower. For this reason species X_1 will be the slow one, whereas species X_2 will be the fast one.

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