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A Theoretical Graph Method for Search and Analysis of Critical Phenomena in Biochemical Systems. I. Graphical Rules for Detecting Oscillators

G. L. Ermakov

Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino 142292, Moscow Region, Russia; fax: (7-0967) 790-553; E-mail: ermakov-gennady@rambler.ru

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Abstract—Sufficient conditions for existence of concentration oscillations of components of complex reactions are considered on the basis of graph theory. Graphical rules were developed for detecting oscillators in kinetic schemes of complex biochemical systems. The main types of topology and principles of construction of kinetic schemes of oscillators containing two, three, and four substances are considered. The resulting oscillators might be a part of the biochemical systems of the n-th order. Under certain conditions they were found to be capable of generating oscillations of the system components.

Key words: graph, oscillations, mathematical simulation

A theoretical graph method for search and analysis of critical phenomena (trigger behavior, multistationary character, and concentration oscillations) in biochemical systems has been developed and described in the preceding work [1]. The goal of this work was to continue this study. The approach suggested in [1] was based on the works of Clark [2-4], Volpert [5], and Ivanova [6-8]. The theoretical graph method allows a reaction/stage/fragment involving two, three, four, etc. substances responsible for critical phenomena to be isolated from the scheme (graph) of complex biochemical reaction. The fragment of the system includes the minimum number of substances and reactions required for the critical phenomena in the system of interest to exist. In this work I consider the main graphical rules of construction of kinetic schemes of biochemical reactions and list the topological types of the possible schemes of oscillators involving two, three, and four substances.

The graph method is an additional tool to methods of qualitative theory of differential equations, which are usually used in analysis of mathematical models of biochemical systems. Illustrative presentation of data is an advantage of the graph method. It is well known that the dynamics of components of a system of reactions is characterized by the values and signs of the roots $\lambda_1, \lambda_2, ..., \lambda_m$ and coefficients $a_1, a_2, ..., a_m$ of the characteristic $\boldsymbol{m}$-th order polynomial of the Jacoby matrix. Based on the graphical rules, it is possible to construct the Jacobian of the system and to determine the values and signs of the coefficients $a_1, a_2, ..., a_m$, which, in turn, determine the values and signs of the roots $\lambda_1, \lambda_2, ..., \lambda_m$. Therefore, the shape of the graph (scheme) of a complex reaction allows information about the type and number of the stationary states of the system to be obtained without solving the corresponding sets of simultaneous differential and algebraic equations.

It should be noted that these are the kinetic equations based on the mass action law that are considered within the framework of the theoretical graph approach. In the absence of significant temperature and spatial inhomogeneity, the system is assumed to be homogeneous.

Any diagram method is secondary with respect to the underlying mathematical apparatus. Diagrams are designed to substitute and simplify analytical procedures. Therefore, the main necessary analytical expressions of chemical kinetics and their representation in a graphical form are worthy of steady and systematic consideration.

1. Basic definitions. Equations of chemical kinetics. Let $X_1, X_2, ..., X_n$ be a list of substances (initial, intermediate, and reaction products) involved in a complex chemical/biochemical reaction composed of $\boldsymbol{R}$ elementary reactions. Here and further reversible stages of the complex reaction are represented by two elementary reactions (direct $\boldsymbol{v_i}$ and back $\boldsymbol{v_{i+1}}$). In this case, the stoichiometric equation of the complex reaction can be written as follows:

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$$\sum_{i=1}^{n}\alpha_{ri}\cdot X_i \xrightarrow{v_r} \sum_{j=1}^{n}\beta_{rj}\cdot X_j \quad (r = 1, 2, ..., R), \tag{1}$$

where $\boldsymbol{v_r}$ is the rate of the $\boldsymbol{r}$-th elementary reaction; α_{ri} and β_{rj} are the nonnegative integer stoichiometric coefficients corresponding to the number of molecules of the initial substance X_i and reaction product X_j involved in one elementary reaction act $\boldsymbol{v_r}$, respectively. According to the mass action law, the rate of the $\boldsymbol{r}$-th elementary reaction of the mechanism (1) can be written as follows:

$$v_r = k_r \cdot x_1^{\alpha_{r1}} \cdot x_2^{\alpha_{r2}} \cdot \ldots \cdot x_i^{\alpha_{ri}}, \tag{2}$$

where k_r, x_i, and α_{ri}, are the reaction rate constant, concentration, and stoichiometric coefficient of the substance X_i, respectively.

By definition, the reaction rate $v^{(X_i)}$ with respect to the component X_i is an algebraic sum of the rates of consumption (***l*** reactions) and production (***p*** reactions) of substance X_i in all elementary reactions v_r ($\boldsymbol{r}$ = 1, 2, ..., $\boldsymbol{S}$, where $\boldsymbol{S = l + p}$) involving X_i, multiplied by corresponding stoichiometric coefficient. The coefficients α_{ir} of the initial substances are assumed to be negative, whereas the product coefficients β_{ir} are assumed to be positive:

$$v^{(X_i)} \equiv \frac{dx_i}{dt} \equiv \dot{x}_i = \sum_{r=1}^{S}(\beta_{ir} - \alpha_{ir})\cdot v_r \quad (i = 1, 2, ..., \boldsymbol{m}). \tag{3}$$

Thus, if certain physicochemical conditions are observed (homogeneity of reaction medium, absence of flows, etc.), any complex chemical/biochemical reaction involving substances X_i (i = 1, 2, ..., ***n***) can be uniquely described by a set of simultaneous ordinary differential equations of the ***m***-th order (***m*** < ***n***). This is true, if some of ***n*** variables are mutually dependent and there are (***n*** – ***m***) balance equations.

It is well known that the time dynamics of the system (3) at low-amplitude fluctuation near a stationary state $\bar{x}_1, \bar{x}_2, ..., \bar{x}_n$ is determined by the roots $\lambda_1, \lambda_2, ..., \lambda_m$ of the characteristic polynomial

$$p(\lambda) = \lambda^m + \lambda^{m-1}\cdot a_1 + \cdots + \lambda^{m-k}\cdot a_k + \cdots + a_m = 0, \tag{4}$$

of the matrix ***B*** (Jacobian), the elements of which are determined, by definition, from the following equation:

$$b_{ij} = \left.\frac{\partial \dot{x}_i}{\partial x_j}\right|_{\bar{x}_1 \ldots \bar{x}_n} \quad (i, j = 1, 2, ..., \boldsymbol{m}). \tag{5}$$

Substitution of expression (3) for derivative $\dot{x}_i$, in which reaction rate $\boldsymbol{v_r}$ is defined in accordance with (2), into Eq. (5) gives an explicit equation for calculation of Jacobian matrix elements:

$$b_{ij} = \sum_{r=1}^{S}(\beta_{ir} - \alpha_{ir})\cdot\alpha_{jr}\cdot\frac{v_r}{x_j} \quad (i, j = 1, 2, ..., \boldsymbol{m}). \tag{6}$$

It follows from the theorems of linear algebra that the coefficient $\boldsymbol{a_1}$ of the characteristic polynomial (4) is equal to the sum of all diagonal elements of matrix ***B*** multiplied by minus one:

$$a_1 = (-1)^1 \cdot \sum_{i=1}^{m} b_{ii}. \tag{7}$$

Coefficients $\boldsymbol{a_2}, \boldsymbol{a_3}, ..., \boldsymbol{a_{m-1}}$ taken with corresponding sign are equal to the sums of all the second order diagonal minors ($M_i = M_{i_1,i_2}^{i_1,i_2}$), the third order diagonal minors ($M_i = M_{i_1,i_2,i_3}^{i_1,i_2,i_3}$), ..., and diagonal minors of order (m – 1) of the Jacobian matrix, respectively ($\boldsymbol{m \times m}$):

$$a_k = (-1)^k \cdot \sum_{i=1}^{l} M_i \quad (k = 2, 3, ..., \boldsymbol{m-1}), \tag{8}$$

where l is the number of all diagonal minors of order ***k*** in the Jacobian matrix, which is equal to the number of combinations of ***m*** elements taken ***k*** at a time. Coefficient $\boldsymbol{a_m}$ is equal to the determinant of the matrix ***B*** taken with corresponding sign:

$$a_m = (-1)^m \cdot \det B. \tag{9}$$

Equations (6)-(9) provide a basis for the development of the diagram method for the search for necessary and sufficient conditions of existence of critical phenomena in complex chemical or biochemical systems.

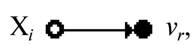
2. Criteria of existence of periodic solutions (oscillations) in mathematical models of biochemical systems. A number of criteria and tests for the presence or absence of periodic solutions of a set of simultaneous differential equations were derived within the framework of the qualitative theory of differential equations. These criteria were derived either on the basis of studies of properties of coefficients $\boldsymbol{a_i}$ (i = 1, 2, ..., ***m***) of the characteristic polynomial (Rauss–Gurvitz test, Clark method [2]), on the basis of studies of properties of the Jacobian matrix elements [9, 10], or on the basis of studies of topological properties of the phase space (tests of Poincare, Bendickson, Dulac, et al.). It was shown in [6, 7] that positive value of leading coefficient $\boldsymbol{a_m}$ and negative value of any of the coefficients $\boldsymbol{a_k}$ ($\boldsymbol{k}$ = 1, 2, ..., ***m*** – **1**) of the characteristic polynomial of order ***m***, i.e., the condition

$$\boldsymbol{a_k} < 0 \text{ and } \boldsymbol{a_m} > 0, \tag{10}$$

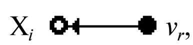
is a sufficient condition of the existence of a periodic solution of a corresponding set of simultaneous differential equations. Conversely, if all coefficients $\boldsymbol{a_i}$ (i = 1, 2, ..., ***m***) are strictly positive at any values of parameters and variables of the set of simultaneous differential equations (i.e., the sign of the function is determined), the stationary point is single and stable, which excludes the existence of periodic solutions.

Equations (6)-(9) and test (10) provide an opportunity to put forward graphical rules of analysis of schemes (graphs) of complex reaction intended to detect and study oscillators in complex biochemical systems. Generally, the results described below are descriptions of the graphical procedure of determination of the sign and value of any of the coefficients of the characteristic polynomial (4) based on the scheme of a complex reaction. Therefore, the rules suggested in this and preceding works are compatible with any existent or newly developed tests of the presence/absence of critical phenomena in any dynamic system, provided that the test is based on analysis of properties of the coefficients $\boldsymbol{a_i}$ (i = 1, 2, ..., $\boldsymbol{m}$) of the characteristic polynomial and/or the properties of the matrix element of the Jacobian $\boldsymbol{b_{ij}}$ (i, j = 1, 2, ..., $\boldsymbol{m}$).

3. Basic definitions. Graphs and graphical elements corresponding to elements of kinetic schemes and kinetic equations. Any complex biochemical reaction (1) can be presented as a two-lobed connected oriented graph. Two-lobed oriented graph consists of nodes of two types and branches of corresponding orientation. The nodes of the first type $X_1, X_2, ..., X_n$ correspond to the substances of the list of reactions (1) and are shown as symbols (O). The nodes of the second type $v_1, v_2, ..., v_R$ correspond to the rates of reactions from the list (1) and are shown as symbols (●). The node of the substance X_i(O) is connected with a branch to the node of the reaction v_r(●) if the substance X_i is a component of the reaction v_r. The branches are directed from the substance node to the reaction rate node



if X_i is one of the initial substances of reaction v_r, or oppositely

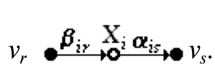


if X_i is a product of the reaction v_r. The branches connecting nodes (O) X_i with nodes (●) v_r are assumed to have weight α_{ir} or β_{ir}, which indicates how many molecules of substance X_i is spent (α_{ir}) or produced (β_{ir}) in an elementary act of the reaction v_r. Further in this work the graph branch is called ***half-pathway.***

Thus, any reaction v_r ($\boldsymbol{r}$ = 1, 2, ..., $\boldsymbol{R}$) of the stoichiometric equation (1) corresponds to a graphically determined element. For example, a reversible stage of exchange between substance X_i surrounded by medium

$$\xrightleftharpoons[\alpha_{is}\cdot v_s]{\beta_{ir}\cdot v_r} X_i$$

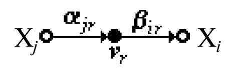
corresponds to two graphical elements (***half-pathways***) belonging to one node X_i, namely, influx of substance X_i to the system with rate v_r and efflux of substance X_i to the surrounding medium with rate v_s:



The reactons of conversion of substance X_j into substance X_i

$$\alpha_{jr}\cdot x_j \xrightarrow{v_r} \beta_{ir}\cdot x_i$$

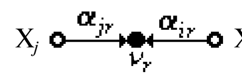
corresponds to the graphical element belonging to the type ***positive pathway***



The reaction of interaction between substances X_j and X_i

$$\alpha_{jr}\cdot x_j + \alpha_{ir}\cdot x_i \xrightarrow{v_r}$$

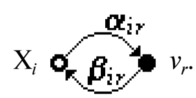
corresponds to the graphical element belonging to the type ***negative pathway***



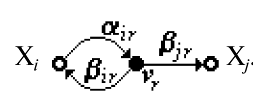
Graphical presentation of autocatalytic reactions, in which the substance is involved both as the initial substance and the product

$$\alpha_{ir}\cdot X_i + \alpha_{jr}\cdot X_j \xrightarrow{v_r} \beta_{ir}\cdot X_i,$$

graphical element ***loop*** should be introduced



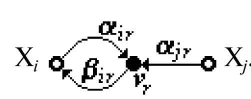
It is well known that neither of the two stoichiometric coefficients of autocatalytic reactions α_{ir} and β_{ir} is equal to zero and $\beta_{ir} > \alpha_{ir}$. The graphical element ***loop*** of a complete graph is contained only as a component of another graphical element (***pathway***). There are two possible cases of such combination. First, loop is a component of a positive pathway



This element incorporates a loop and a positive pathway directed from substance X_i to substance X_j. This graphical element formally corresponds to the following scheme:

$$\alpha_{ir}\cdot X_i \xrightarrow{v_r} \beta_{ir}\cdot X_i + \beta_{jr}\cdot X_j \quad .$$

Second, loop is a component of a negative-positive pathway



This graphical element includes a loop, a positive pathway, and a negative pathway. If the stoichiometric coefficients are equal to $\alpha_{ir} = \alpha_{jr} = 1$ and $\beta_{ir} = 2$, this element corresponds to the following autocatalytic reaction:

$$X_i + X_j \xrightarrow{v_r} 2 \cdot X_i \ .$$

The values of the stoichiometric coefficients in the pathways including the graphical element loop are determined by the law of conservation of matter. It will be shown below in this work that the graphical element loop is incorporated in graphs of virtually all existing models of both hypothetical and experimentally known oscillators in chemical systems.

Graphs of complex reactions may contain closed sequences (cycles) of positive and/or negative pathways combining $\boldsymbol{k}$ nodes of substances X_i (i = 1, 2, ..., k). Within the framework of the terminology suggested by Clark, let the cycle be called ***even-numbered*** if it contains **(a)** only positive pathways or **(b)** only an even number of negative pathways or **(c)** an even number of negative pathways plus any number of positive pathways. The cycles without characters **(a-c)** are called ***odd-numbered***. An individual negative pathway is an even-numbered cycle.

Volpert [5] showed that the set of simultaneous differential equations (3) corresponding to the list of reactions (1) could be represented by a complete two-lobed connected oriented graph. In this case, there is an illustrative graphical presentation of equations for calculation of Jacobian matrix elements (6) and coefficients of characteristic polynomial (7)-(9), because they correspond to a certain part of the complete graph of the system (1). Moreover, the graphical rules can be suggested to evaluate which parts of the complete graph of the system determine the signs and values of the coefficients $\boldsymbol{a_k}$ (k = 1, 2, ..., $\boldsymbol{m}$) of the characteristic polynomial (4).

Each element $\boldsymbol{b_{ij}}$ (i, j = 1, 2, ..., $\boldsymbol{m}$) of the Jacoby matrix (6) corresponds to a certain part of the complete graph of the initial system (1). **The diagonal element $\boldsymbol{b_{ii}}$ corresponds to all half-pathways passing out of the substance $\boldsymbol{X_i}$. Element $\boldsymbol{b_{ij}}$ corresponds to all pathways (positive and/or negative) leading from substance $\boldsymbol{X_j}$ to substance $\boldsymbol{X_i}$ (Rule 1)**. Therefore, the Jacobian of the system can be constructed as the complete graph of the system. Equation (6) in this case determines the value and sign of the graphical elements listed above as components of complete graph (***half-pathway***, ***pathway***, ***loop***, ***and cycle***) (table). It follows from the table that half-pathway, positive pathway, and negative pathway are characterized by negative, positive, and negative values, respectively. Even-numbered and odd-numbered cycles have positive and negative values, respectively. In this case, if $\alpha_{ir} = \beta_{ir}$, or $\alpha_{ir} > \beta_{ir}$, or $\alpha_{ir} < \beta_{ir}$, the numerical value of the graphical element loop is zero, or negative, or positive, respectively (table). Let the three values of the matrix element $\boldsymbol{b_{ii}}$ be brought in correspondence with three graphical elements: ***(0)-loop*** ($\alpha_{ir} = \beta_{ir}$), ***(–)-loop*** ($\alpha_{ir} > \beta_{ir}$), and ***(+)-loop*** ($\alpha_{ir} < \beta_{ir}$), respectively.

The graphical representation of the diagonal minor of order $\boldsymbol{k}$ ($\boldsymbol{k}$ = 2, 3, ..., $\boldsymbol{m}$ **– 1**) in Eq. (8) is an aggregate of all pathways (both negative and positive) combining $\boldsymbol{k}$ substances of the system and all half-pathways/loops emerging from all nodes of substances of the system. The graphical representation of the Jacobian determinant or coefficient $\boldsymbol{a_m}$ is a connected part of the complete graph of the system (3), which incorporates substances X_i (i = 1, 2, ..., $\boldsymbol{m}$). According to the rule of expansion of determinants, the expansion of the minor of order $\boldsymbol{k}$ gives an algebraic sum of the products of the Jacobian matrix elements. Each product containing exactly $\boldsymbol{k}$ factors has a graphic equivalent, **subgraph** of order $\boldsymbol{k}$.

Subgraph of order $\boldsymbol{k}$ is an aggregate (product) of half-pathways/loops, or half-pathways/loops and cycles, or only cycles. In each subgraph the substance node is the initial point of the only one half-pathway/loop or cycle. The minimal connected part of the graph combining $\boldsymbol{k}$ substance nodes and $\boldsymbol{k}$ reaction nodes is called **graph** of order $\boldsymbol{k}$**.** Each graph of order $\boldsymbol{k}$ includes several **subgraphs** of order $\boldsymbol{k}$. In other words, in graph of order $\boldsymbol{k}$ any substance node X_i, can be represented by any degree (d_i is the number of half-ways emerging from the node), whereas in subgraph of order $\boldsymbol{k}$ the node X_i (i = 1, 2, ..., k) may have degree equal to only one.

Thus, taking into account the definitions given above, the coefficient $\boldsymbol{a_k}$ ($\boldsymbol{k}$ = 1, 2, ..., $\boldsymbol{m}$) of the characteristic polynomial (4) is an algebraic sum of subgraphs of order $\boldsymbol{k}$ of the selected aggregate of $\boldsymbol{k}$ substances and $\boldsymbol{k}$ reactions. **The sign of the coefficient $\boldsymbol{a_k}$ is determined by three factors. First, the sign of the coefficient in Eqs. (7)-(9). Second, the sign of each product of the Jacobian matrix elements, which is determined by the rules of expansion of determinants. Finally, the sign of each element $\boldsymbol{b_{ij}}$ determined in accordance with Eq. (6) (table) (Rule 2).**

Let the complete graph or its arbitrary part be denoted as $\boldsymbol{G_i}$. Let a fragment or a part of the complete graph $\boldsymbol{G_i}$ be denoted with a double-subscript symbol, i.e., $\boldsymbol{G_{ij}}$, where i is the ordinal number of the complete graph; j is the ordinal number of the fragment of the complete graph $\boldsymbol{G_i}$. Let the graph of order $\boldsymbol{k}$ be denoted as ${}^{k}\boldsymbol{G_i}$, and its constituting subgraphs, as $\boldsymbol{g_{ij}}$.

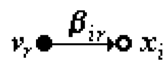
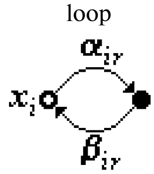
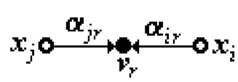
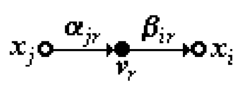
RESULTS AND DISCUSSION

Using the rules of expansion of determinants and graphical correspondence of each product of Jacobian element, it can be shown that coefficient $\boldsymbol{a_k}$ ($\boldsymbol{k}$ = 1, 2, ..., $\boldsymbol{m}$) is an algebraic sum of subgraphs of order $\boldsymbol{k}$ of three types in the selected aggregate of substances X_{i_1}, X_{i_2}, ..., X_{i_k}: aggregate (product) of only half-pathways and/or loops ($g_n^{(I)}$); only one cycle or aggregate of cycles ($g_n^{(II)}$, $g_n^{(IV)}$); product of half-pathways and/or loops and cycles ($g_n^{(III)}$, $g_n^{(V)}$).

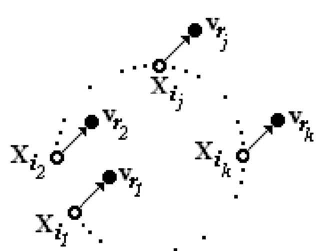
Subgraph $g_n^{(I)}$ is an aggregate of all half-pathways and/or loops emerging only one time from nodes X_{i_1}, X_{i_2}, ..., X_{i_k}. Subgraph $g_n^{(I)}$ is **positive** if it contains: a) only half-pathways; b) half-pathways and an even number of elements (+)-loop; c) half-pathways and any number of ele-

Value and sign of graphical elements half-pathway, loop, positive pathway, and negative pathway constituting a complex reaction graph. Correspondence between graphical elements and matrix elements of Jacobian $\|b_{ij}\|$:

$$b_{ij} = \frac{\partial \dot{x}_i}{\partial x_j} = \sum_{r=1}^{S} (\beta_{ir} - \alpha_{ir}) \cdot \alpha_{jr} \cdot \frac{v_r}{x_j} \qquad (i, j = 1, \ldots, m)$$

Stoichiometric equation of reaction	Graphical representation of reaction	Rate of changes of concentration x_i of substance X_i	Kinetic equation of the reaction rate (v_r) calculated from the mass action law	Value and sign of graphical element corresponding to matrix elements b_{ii} and b_{ij}
$\alpha_{ir} \cdot X_i \xrightarrow{v_r}$	half-pathway 	$\dot{x}_i = -\alpha_{ir} \cdot v_r$	$v_r = k_r \cdot x_i^{\alpha_{ir}}$	$b_{ii} = -(\alpha_{ir})^2 \cdot \frac{v_r}{x_i}$
$\xrightarrow{v_r} \beta_{ir} \cdot X_i$	half-pathway 	$\dot{x}_i = \beta_{ir} \cdot v_r$	$v_r = k_r$	$b_{ii} = 0$
$\alpha_{ir} \cdot X_i \xrightarrow{v_r} \beta_{ir} \cdot X_i$	loop 	$\dot{x}_i = -\alpha_{ir} \cdot v_r + \beta_{ir} \cdot v_r$	$v_r = k_r \cdot x_i^{\alpha_{ir}}$	$b_{ii} = \alpha_{ir} \cdot (\beta_{ir} - \alpha_{ir}) \cdot \frac{v_r}{x_i}$
$\alpha_{jr} \cdot X_j + \alpha_{ir} \cdot X_i \xrightarrow{v_r}$	negative pathway 	$\dot{x}_i = -\alpha_{ir} \cdot v_r$	$v_r = k_r \cdot x_j^{\alpha_{jr}} \cdot x_i^{\alpha_{ir}}$	$b_{ij} = -\alpha_{ir} \cdot \alpha_{jr} \cdot \frac{v_r}{x_j}$
$\alpha_{jr} \cdot X_j \xrightarrow{v_r} \beta_{ir} \cdot X_i$	positive pathway 	$\dot{x}_i = \beta_{ir} \cdot v_r$	$v_r = k_r \cdot x_j^{\alpha_{jr}}$	$b_{ij} = +\alpha_{jr} \cdot \beta_{ir} \cdot \frac{v_r}{x_j}$

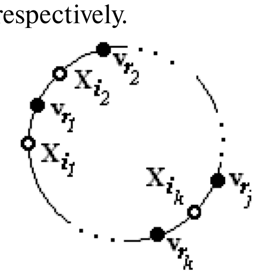
ments (–)-loop. Subgraph $g_n^{(I)}$ is **negative** if it contains half-pathways and an odd number of elements (+)-loop:



$\mathbf{g_i^{(I)}}$ **(i = 1, 2, ..., p)**

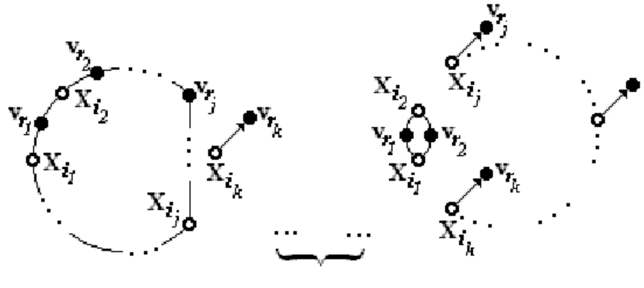
where $p = \prod_{l=1}^{k} d_{i_l}$ is the number of subgraphs of type $g_n^{(I)}$ of the selected aggregate of the substance nodes $X_{i_1}, X_{i_2}, \ldots, X_{i_k}$, each of them having the power equal to d_{i_1}.

Subgraphs $g_n^{(II)}$ represent all cycles combining substances $X_{i_1}, X_{i_2}, \ldots, X_{i_k}$ of the selected aggregate taken two times if the direction of the pathways coincides with the direction of the clockwise and counter-clockwise circumvention of a cycle. The subgraph of this type is **negative** or **positive** if it consists of an even-numbered or an odd-numbered cycle, respectively.



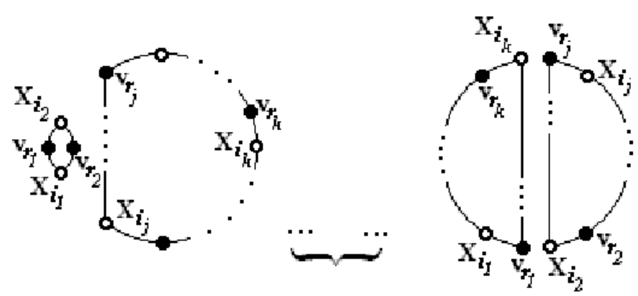
$\mathbf{g_n^{(II)}}$

Subgraphs $g_n^{(III)}$ represent the products of cycles over 2, 3, ..., $\boldsymbol{k}-1$ substances multiplied by half-pathways and/or loops emerging from the other substances $\boldsymbol{k}-2$, $\boldsymbol{k}-3$, ..., 1 of the selected aggregate. Subgraph $g_n^{(III)}$ is **negative** if it consists of a product of: a) even-numbered cycle and half-pathways/(–)-loop; b) odd-numbered cycle, half-pathways, and an odd number of elements (+)-loop. Subgraph $g_n^{(III)}$ is **positive** if it consists of a product of: a) an even-numbered cycle, half-pathways, and an odd number of elements (+)-loop; b) an odd-numbered cycle and half-pathways/(–)-loop.



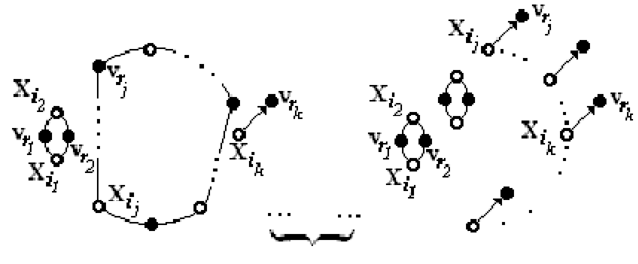
$g_n^{(III)}$

Subgraphs $g_n^{(IV)}$ represent aggregates of two, three, etc. cycles combining two, three, ..., $\boldsymbol{k}-2$ substances of the selected aggregate. Subgraph $g_n^{(IV)}$ is **negative** if it consists of an odd number of even numbered cycles and it is **positive** if: a) all constituting cycles are even-numbered; b) subgraph contains an even number of odd-numbered cycles.



$g_n^{(IV)}$

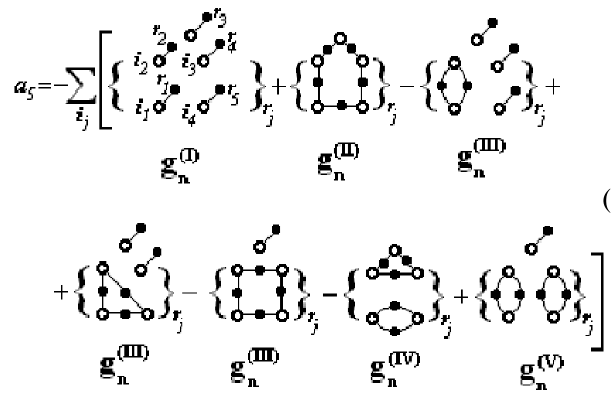
Subgraphs $g_n^{(V)}$ represent the product of a cycle of two, three, ..., $\boldsymbol{k}-j$ substances multiplied by half-pathways/loops emerging from the substances that are not combined into cycles. Subgraph $g_n^{(V)}$ is **negative** if it contains: a) an odd number of even-numbered cycles and half-pathways/(–)-loops; b) all its cycles are odd-numbered; c) an odd number of even-numbered cycles, half-pathways, and an odd number of elements (+)-loop. This subgraph is **positive** if contains: a) only even-numbered cycles and any number of half-pathways and elements (–)-loop; b) an even number of odd-numbered cycles and any number of half-pathways and elements (–)-loop; c) only even-numbered cycles, half-pathways, and an even number of elements (+)-loop; d) an even number of odd-numbered cycles, half-pathways, and an even number of elements (+)-loop; e) an odd number of odd-numbered cycles, half-pathways, and an odd number of elements (+)-loop.



$g_n^{(V)}$

Any of subgraphs $g_n^{(I)}$, $g_n^{(III)}$, and $g_n^{(V)}$ is **equal to** zero if it contains at least one element (0)-loop.

As an example, let the expression for calculation of coefficient $\boldsymbol{a}_5$ be written as an algebraic sum of subgraphs:



(11)

Expression (11) contains all types of subgraphs listed above. The sum is taken negative in accordance with definition (8), signs in squared brackets are determined by the rule of expansion of determinants. The final value of each subgraph in expression (11) is determined by the values of components of their graphical elements (table). Thus, the sign and value of coefficient $\boldsymbol{a}_k$ can be determined by exhaustive search of all subgraphs of order $\boldsymbol{k}$ constituting the coefficient.

In studies of mechanisms/graphs of complex biochemical systems it is useful to consider separately the minimal connected part (i.e., graph of order $\boldsymbol{k}$) of the complete graph, which determines the negative sign of the coefficient $\boldsymbol{a}_k$ ($\boldsymbol{k}$ = 1, 2, ..., $\boldsymbol{m}$ – **1**). Let a graph of order $\boldsymbol{k}$ be **negative** if it gives a negative contribution to coefficient $\boldsymbol{a}_k$. The value of the negative graph of order $\boldsymbol{k}$ is determined by values of stoichiometric coefficients, rates $\boldsymbol{v}_r$, and concentrations $\boldsymbol{x}_i$ in a stationary state. These values can be calculated from Eq. (6).

Thus, the problem of determination of signs of coefficients $\boldsymbol{a_k}$ ($\boldsymbol{k}$ = 1, 2, ..., $\boldsymbol{m} - \mathbf{1}$) and $\boldsymbol{a_m}$ (i.e., sufficient conditions of existence of oscillations (10)) is reduced to the search for the topology of the minimal **negative** graph of order $\boldsymbol{k}$ (oscillator) in the complete graph of the system and to the elucidation of the complete graph topology providing a positive value of the coefficient $\boldsymbol{a_m}$. Let us compile a list of the main types of topology of the minimal negative graphs of orders 1, 2, 3, and 4, which give a negative contribution to coefficients $\boldsymbol{a_1}$, $\boldsymbol{a_2}$, $\boldsymbol{a_3}$, and $\boldsymbol{a_4}$.

1. Value and sign of coefficient $\boldsymbol{a_1}$. It follows from Eq. (7) and Rule 1 that coefficient $\boldsymbol{a_1}$ is graphically represented by an aggregate of all half-pathways and loops emerging from the substance node X_i (i = 1, 2, ..., $\boldsymbol{m}$) of the complete graph of the system. In other words, coefficient $\boldsymbol{a_1}$ is an algebraic sum of the subgraphs of the first order of the type $g_n^{(I)}$, in which each substance X_i (i = 1, 2, ..., $\boldsymbol{m}$) is the initial point of only one half-pathway or loop:

$$a_I = \sum_{n} \mathbf{g}_{\mathbf{n}}^{(\mathbf{I})} = -\sum_{i=1}^{m} \left[\left\{ X_i \!\circ\!\!\longrightarrow\!\bullet\, v_r \right\}_r \right] \quad (r = 1, 2, ..., d_i), \qquad (12)$$

where d_i is the node X_i degree, i.e., the number of all half-pathways/loops emerging from the node. On the basis of Eq. (7) and definition of matrix elements $\boldsymbol{b_{ii}}$ (table) it is possible to write the equations for calculating the value and sign of coefficient $\boldsymbol{a_1}$.

If the complete graph of the system **does not contain a graphical elements loop**, the coefficient $\boldsymbol{a_1}$ at any values of stoichiometric coefficients and system parameters is **strictly positive** and equal to:

$$a_1 = -\sum_{i=1}^{m} b_{ii} = \sum_{i=1}^{m} \sum_{r=1}^{d_i} \alpha_{ir}^2 \cdot \frac{v_r}{x_i} > 0. \qquad (13)$$

If a given node X_k of the complete graph of the system **contains at least one graphical element loop** ($X_k \underset{\beta_{ir}}{\overset{\alpha_{ir}}{\rightleftarrows}} v_r$,), the coefficient $\boldsymbol{a_1}$ contains a negative component determined by this element, and its value is equal to:

$$a_1 = -\sum_{i=1}^{m} b_{ii} = \sum_{i=1}^{k-1} \sum_{r=1}^{d_i} \alpha_{ir}^2 \cdot \frac{v_r}{x_i} - \alpha_{ks} \cdot (\beta_{ks} - \alpha_{ks}) \cdot \frac{v_s}{x_k} + \sum_{i=k+1}^{m} \sum_{r=1}^{d_i} \alpha_{ir}^2 \cdot \frac{v_r}{x_i}. \qquad (14)$$

It follows from Eq. (14) that if the element at the node X_k is **(0)-loop** ($\beta_{ks} = \alpha_{ks} = 1$) or **(–)-loop** ($\beta_{ks} < \alpha_{ks}$), inequality $\boldsymbol{a_1} > 0$ is valid at any values of system parameters. If there is an element **(+)-loop** ($\beta_{ir} > \alpha_{ir}$) at the node X_k, the coefficient $\boldsymbol{a_1}$ at certain values of system parameters may assume a **negative**, a positive, or zero value. Therefore, it follows from Eqs. (12)-(14) that **coefficient $\boldsymbol{a_1}$ at certain values of system parameters takes a negative if and only if the complete graph of the system contains at least one graphical element (+)-loop. Otherwise, coefficient $\boldsymbol{a_1}$ is positive or equal to zero (Rule 3)**.

On the basis of graphical Rule 3 for coefficient $\boldsymbol{a_1}$ it is possible to find graphical equivalents of well-known tests of the absence of a limiting cycle in simply connected two-dimensional phase space (Dulac–Bendickson test) or tests of the presence of a limiting cycle in a doubly connected domain of two-dimensional phase space (Poincare test). It is well known that a periodic solution of the second order set of simultaneous equations corresponds either to a stationary point of the type center (i.e., roots λ_1, λ_2 of the characteristic polynomial are imaginary) or to the existence of an attractor of the limiting cycle type, which encircles at least one unstable stationary point. It is also well known that in case of specific point of type center the following expressions are valid: $\boldsymbol{a_1} = 0$ and $\boldsymbol{a_2} > 0$. On the other hand, according to the Poincare–Dulac–Bendickson test, alternating-sign value of coefficient $\boldsymbol{a_1}$ and positive value of coefficient $\boldsymbol{a_2}$ are a sufficient condition of existence of a limiting cycle.

Thus, **if the graph of the complex reaction, whose dynamics is described by the second order set of simultaneous differential equations, does not contain a graphical elements loop, none of the system parameters or variables correspond to a periodic solution of the set of simultaneous differential equations (Rule 4)**. An alternating-sign value of coefficient $\boldsymbol{a_1}$ can be obtained only in case of the presence of positive and negative components in the equation for calculation of coefficient (12). It follows from Eq. (14) that a positive component is due to the presence of half-pathways or element (–)-loop, whereas a negative component is due to the presence of autocatalytic stages (graphical element (+)-loop) in the system. In other words, if the graph of the reaction system does not contain element (+)-loop, coefficient $\boldsymbol{a_1} > 0$. If condition $\boldsymbol{a_2} > 0$ is met, the two-dimensional system contains only one stationary point of the type asymptotically stable center/focus. If the complete graph of the system contains an element loop but coefficient $\boldsymbol{a_1}$ is strictly negative throughout the whole area of changes of parameters and variables of the system, and condition $\boldsymbol{a_2} > 0$ is observed, the system has an unstable stationary point belonging to the type node/focus.

2. Value and sign of coefficient $\boldsymbol{a_2}$. Minimal negative graphs (oscillators) of the second order. According to Eq. (8), coefficient $\boldsymbol{a_2}$ is a sum of all diagonal minors of the second order of Jacobian (6). The graphical representation of second order minor is a fraction of the complete graph of the system including two substance nodes X_i, X_j and all half-pathways (and/or loops) emerging from the nodes X_i and X_j, as well as all pathways connecting the two nodes. According to the rule of expansion of determinants, any second order minor of Jacobian (6) is equal to the algebraic sum of the products of pairs of the Jacobian elements of two types: positive ($+\boldsymbol{b_{ii}b_{jj}}$) and negative ($-\boldsymbol{b_{ij}b_{ji}}$). Thus, coefficient $\boldsymbol{a_2}$ can be graphically represented by an aggregate of all subgraphs of the second order of two types. The first type ($g_{n_1}^{(I)}$) is represented by the products of pairs of all half-pathways (and loops) emerging from the nodes X_i and X_j and taken from the rules of expansion of determinants with a plus sign. The second type ($g^{(II)}_{n_2}$) is represented by the products of pairs of all pathways connecting the nodes X_i and X_j, i.e., the cycles taken from the rules of expansion of determinants with a minus sign. The negative pathway $X_j \circ\!\xrightarrow{\alpha_{jr}}\!\bullet_{v_r}\!\xleftarrow{\alpha_{ir}}\!\circ\, X_i$ is assumed to be an even-numbered cycle numerically equal to the positive product of two negative pathways, which is signed minus in accordance with the rules of expansion of determinants:

$$g_n^{(II)} = -\alpha_{ir}^2 \cdot \alpha_{jr}^2 \frac{v_r \cdot v_r}{x_j \cdot x_i}. \quad (15)$$

Coefficient a_2 in a symbolic form can be written as:

$$a_2 = \sum_{n_1, n_2} (g^{(I)}_{n_1} + g^{(II)}_{n_2}) = +\sum_{i,j} \left[\left\{ \begin{matrix} X_i \circ\!-\!\bullet v_r \\ X_j \circ\!-\!\bullet v_s \end{matrix} \right\}_{r,s} - \left\{ v_r \bullet \!\!\begin{matrix} \circ X_i \\ \bullet v_s \\ \circ X_j \end{matrix} \right\}_{r,s} \right]. \quad (16)$$

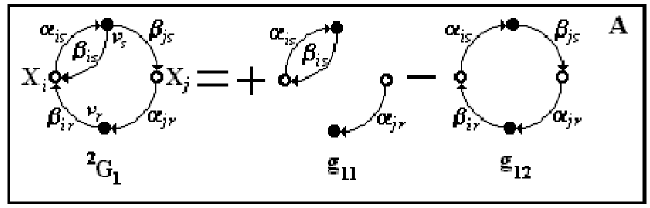
If the complete graph of the system **contains no loops**, whereas **each stoichiometric coefficient is equal to one**, the negative contribution to coefficient a_2, in accordance with Eq. (16), can be provided only by even-numbered cycles. However, if the conditions listed above are observed, the even-numbered cycle subgraph is always cancelled with the subgraph composed of the products of the half-pathways belonging to the given even-numbered cycle (these subgraphs have equal values but opposite signs). Therefore, at all values of system parameters the following inequality is observed: **coefficient $a_2 \geq 0$**. If the complete graph of the system **contains a graphical element (0)-loop/(+)-loop and/or if there are stages (graph branches) with stoichiometric coefficients larger than one**, there should be the second order subgraphs providing a negative contribution to coefficient a_2, whereas at certain system parameters coefficient a_2 may take negative values (**Rule 5**).

All possible variants of the topology of second order negative graphs fall into four groups (schemes **A-D**). The first and the second groups (schemes **A** and **B**) are graphs $^2G_1$ and $^2G_2$ produced by an even-numbered cycle consisting of positive pathways and one or two loops. The third group (scheme **C**) consists of graphs $^2G_3$ produced by an even-numbered cycle that incorporates only positive pathways. The fourth group (scheme **D**) consists of graphs $^2G_4$ produced by an odd-numbered cycle and a loop included in a negative pathway. The numerical value of any negative graph of the second order is:

$$^2G_n = K_{^2G_n} \cdot \frac{v_r \cdot v_s}{x_i \cdot x_j}, \quad (17)$$

where $K_{^2G_n}$ is the algebraic sum of the products of the stoichiometric coefficients of the second order subgraphs g_{ni} ($i = 1, 2, ..., l$) constituting the graph ^2G_n. The values of all negative subgraphs differ from each other only by the value of the coefficient $K_{^2G_n}$, which is determined by the topology of each graph ^2G_n.

Scheme **A** shows graph $^2G_1$ and two its subgraph components g_{11}, g_{12}.



Coefficient $K_{^2G_1}$ of graph $^2G_1$ is equal to:

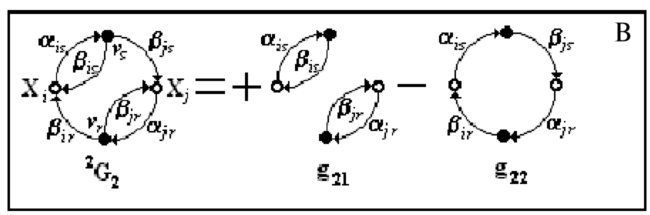
$$K_{^2G_1} = +K_{g_{11}} - K_{g_{12}} = \alpha_{is} \cdot (\beta_{is} - \alpha_{is}) \cdot (-\alpha_{jr}^2) - \alpha_{is} \cdot \beta_{js} \cdot \alpha_{jr} \cdot \beta_{ir}. \quad (18)$$

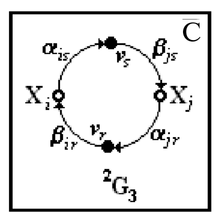
It follows from Eq. (18) that graph $^2G_1$ is negative, i.e., coefficient $K_{^2G_1}$ is negative if the following conditions are met:

$$\beta_{is} \geq \alpha_{is}, \quad (19)$$

$$(\alpha_{is} - \beta_{is}) < \frac{\beta_{js} \cdot \beta_{ir}}{\alpha_{jr}} \quad (\text{at } \beta_{is} < \alpha_{is}). \quad (20)$$

Scheme **B** shows graph $^2G_2$ and two its subgraph components g_{21}, g_{22}.





Coefficient $K_{^2G_2}$ of graph $^2G_2$ is equal to:

$$K_{^2G_2} = \alpha_{is} \cdot (\beta_{is} - \alpha_{is}) \cdot \alpha_{jr} \cdot (\beta_{jr} - \alpha_{jr}) - \alpha_{is} \cdot \beta_{js} \cdot \alpha_{jr} \cdot \beta_{ir}. \quad (21)$$

Coefficient $K_{^2G_2}$ is negative if at least one of the two following equations is valid, $\beta_{is} = \alpha_{is}$ and $\beta_{jr} = \alpha_{jr}$, or if the inequalities $\beta_{is} < \alpha_{is}$ and $\beta_{jr} > \alpha_{jr}$ (or vice versa) are observed. If the two product stoichiometric coefficients are larger or smaller than pair α_{is} and α_{jr}, coefficient $K_{^2G_2}$ is negative, provided the following inequality is observed:

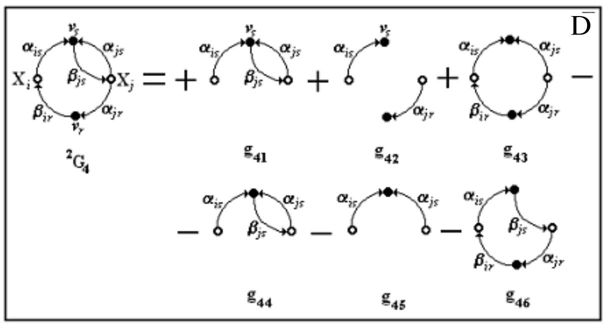
$$(\beta_{is}-\alpha_{is})\cdot(\beta_{jr}-\alpha_{jr})<\beta_{js}\cdot\beta_{ir}. \quad (22)$$

Scheme **C** shows graph $^2\boldsymbol{G}_3$, whose coefficient (23) $K_{^2G_n}$ is negative if condition (24) is valid:

$$K_{^2G_3}=(-\alpha_{is}^2)\cdot(-\alpha_{jr}^2)-\alpha_{is}\cdot\beta_{js}\cdot\alpha_{jr}\cdot\beta_{ir}, \quad (23)$$

$$\alpha_{is}\cdot\alpha_{jr}<\beta_{js}\cdot\beta_{ir}. \quad (24)$$

Scheme **D** shows graph $^2\boldsymbol{G}_4$ and its subgraph components: $\boldsymbol{g}_{41}$, $\boldsymbol{g}_{42}$, and $\boldsymbol{g}_{43}$ (a product of a half-pathway multiplied by a loop, a product of two half-pathways multiplied by one another, and an odd-numbered cycle, respectively); $\boldsymbol{g}_{44}$ (an even-numbered cycle including substances X_i and X_j with counter-clockwise circumvention of the cycle); $\boldsymbol{g}_{45}$ (an even-numbered cycle); $\boldsymbol{g}_{46}$ (an even-numbered cycle of positive pathways).



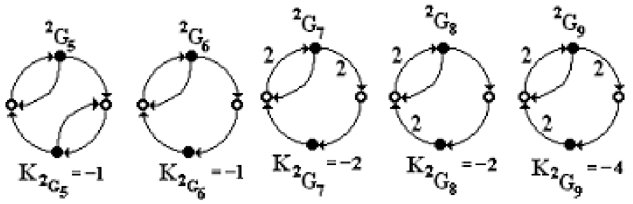
The algebraic sum of the subgraphs $\boldsymbol{g}_{41}$, $\boldsymbol{g}_{44}$, and $\boldsymbol{g}_{45}$ is equal to zero. It should be noted that subgraphs containing at least two identical subscripts of reaction rate are cancelled out. Summation of all subgraphs of scheme **D** with necessary cancellations gives the following equation for calculation of coefficient $K_{^2G_4}$:

$$K_{^2G_4}=\alpha_{is}^2\cdot\alpha_{jr}^2+\alpha_{is}\cdot\alpha_{js}\cdot\alpha_{jr}\cdot\beta_{ir}-\alpha_{is}\cdot\beta_{js}\cdot\alpha_{jr}\cdot\beta_{ir}. \quad (25)$$

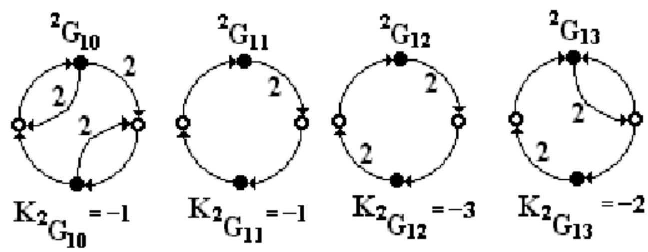
Coefficient $K_{^2G_4}$ is negative if the following inequality is valid:

$$\beta_{js}>\alpha_{js}+\frac{\alpha_{is}\cdot\alpha_{jr}}{\beta_{ir}}. \quad (26)$$

Usually, the stoichiometric coefficients in biochemical systems are equal to 1 or 2. In the simplest case, i.e., if all stoichiometric coefficients are equal to one, the first and the second groups (schemes **A** and **B**) are characterized by the existence of only one type of topology of the minimal negative graph of the second order each: graphs $^2\boldsymbol{G}_5$ and $^2\boldsymbol{G}_6$.



If the stoichiometric coefficients can take the values 1 and 2, all possible variants of topology of the minimal negative graph of the second order can be derived from Eqs. (18)-(26). Let us consider the following graphs as an example: three variants of negative graphs of the first group (scheme **A**)—$^2\boldsymbol{G}_7$, $^2\boldsymbol{G}_8$, $^2\boldsymbol{G}_9$; one graph of the second group (scheme **B**)—$^2\boldsymbol{G}_{10}$; two graphs of the third group (scheme **C**)—$^2\boldsymbol{G}_{11}$, $^2\boldsymbol{G}_{12}$, and one graph of the fourth group (scheme **D**)—$^2\boldsymbol{G}_{13}$.



A specific feature of the graphs containing graphical element (+)-loop (autocatalytic reaction) is that this graphical element is also a negative component of coefficient $\boldsymbol{a}_1$. Therefore, **coefficient $\boldsymbol{a}_2$ is negative only if the complete graph of the system contains at least one graphical element (0)-loop/(+)-loop, or a half-pathway of degree $\beta_{ir}>1$ in the cycle including two substances, or a combination of these elements. In any other cases coefficient $\boldsymbol{a}_2$ is positive or equal to zero (Rule 6).** Rule 6 can be recast as follows. **If a complete graph of a system does not contain loops and all stoichiometric coefficients are equal to one, coefficient $\boldsymbol{a}_2\geq 0$ at any values of system parameters (Rule 6.1).**

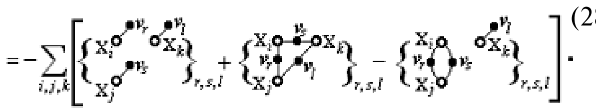
3. Value and sign of coefficient $\boldsymbol{a}_3$. Minimal negative graphs (oscillators) of the third order. Coefficient $\boldsymbol{a}_3$ is the sum of all the third order diagonal minors of the initial system Jacobian of order $\boldsymbol{m}$. By definition, the third order determinant is equal to the algebraic sum of six products of three elements of the determinant selected in accordance with a corresponding rule. For instance, diagonal minor $M^{i_1,i_2,i_3}_{i_1,i_2,i_3}$ is equal to:

$$M^{i_1,i_2,i_3}_{i_1,i_2,i_3}=b_{i_1i_1}b_{i_2i_2}b_{i_3i_3}+b_{i_2i_1}b_{i_3i_2}b_{i_1i_3}+b_{i_3i_1}b_{i_2i_3}b_{i_1i_2}-b_{i_1i_1}b_{i_2i_3}b_{i_3i_2}-b_{i_2i_2}b_{i_1i_3}b_{i_3i_1}-b_{i_3i_3}b_{i_1i_2}b_{i_2i_1}. \quad (27)$$

Because each Jacobian element has a simple graphical representation (table), the product $b_{i_1i_1}b_{i_2i_2}b_{i_3i_3}$ corresponds to all half-pathways, which are incident one time to each substance node X_{i1}, X_{i2}, and X_{i3}. Products $b_{i_2i_1}b_{i_3i_2}b_{i_1i_3}$ and $b_{i_3i_1}b_{i_2i_3}b_{i_3i_2}$ correspond to the cycles passing through the three substances. In the former case the cycle passes through the substances in the direction from X_{i1} to X_{i3}, whereas in the latter case, in opposite direction. The products signed minus (according to the rule of expansion of determinants), $b_{i_1i_1}b_{i_2i_3}b_{i_3i_2}$, $b_{i_2i_2}b_{i_1i_3}b_{i_3i_1}$, and $b_{i_3i_3}b_{i_1i_2}b_{i_2i_1}$, represent the half-pathway emerging from substance X_1 and a cycle passing through substances X_{i2} and X_{i3} in case of the first product, etc. Taking into account the sign of the coefficient $\boldsymbol{a}_3$

in Eq. (8) and signs assigned as a result of expansion of the third order minors, it should be concluded that only positive subgraphs give a negative contribution to the coefficient, i.e., the coefficient a_3 in a symbol form can be written as follows:

$$a_3 = \sum_{n_1, n_2, n_3} (g^{(I)}_{n_1} + g^{(II)}_{n_2} + g^{(III)}_{n_3}) =$$

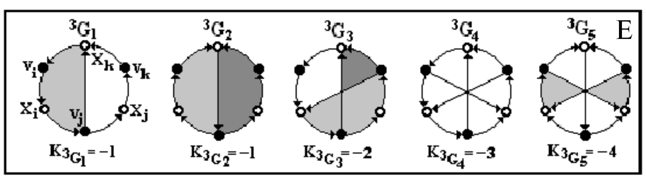


Let the third order negative graphs and the main graphical rules of their presentation be recalled once more. The value of any third order negative graph is:

$$^3G_n = K_{^3G_n} \cdot \frac{v_i \cdot v_j \cdot v_k}{x_i \cdot x_j \cdot x_k}, \quad (29)$$

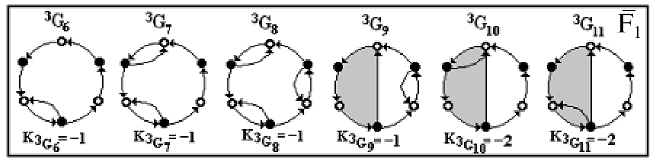
where coefficient $K_{^3G_n} = -1, -2, ..., -l$ is determined by the values of stoichiometric coefficients and graph topology.

Scheme **E** shows the group of all third order negative graphs that meet the following condition: if the degree of any substance node is one, the third order negative graphs contain only **positive pathways** with the stoichiometric coefficient equal to one. For the sake of illustrative presentation, here and further in schemes the cycles involving two and three substances are shaded gray.



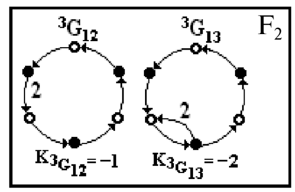
The third order graph is negative if it contains at least **two even-numbered cycles** involving substances X_i, X_j, and X_k, which are required to compensate the positive contribution of the only subgraph composed of a product of half-pathways. Graphs $^3\boldsymbol{G}_1$ and $^3\boldsymbol{G}_2$ contain two even-numbered cycles each. In graph $^3\boldsymbol{G}_1$ one cycle includes two substances, X_i and X_k, whereas another cycle includes the three substances. Graph $^3\boldsymbol{G}_2$ consists of two even-numbered cycles including two substances each. Graphs $^3\boldsymbol{G}_3$, $^3\boldsymbol{G}_4$, and $^3\boldsymbol{G}_5$ contain three, four, and five even-numbered cycles, respectively, all cycles of graph $^3\boldsymbol{G}_5$ containing only two substances.

Schemes $\mathbf{F_1}$ and $\mathbf{F_2}$ show a group of graphs containing only positive pathways and loops, the stoichiometric coefficients of all pathways of the graphs shown in scheme $\mathbf{F_1}$ being equal to one. Graphs $^3\boldsymbol{G}_6$, $^3\boldsymbol{G}_7$, and $^3\boldsymbol{G}_8$ contain one, two, and three loops, respectively, each loop being incorporated in a cycle of positive pathways.

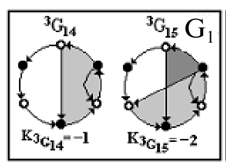


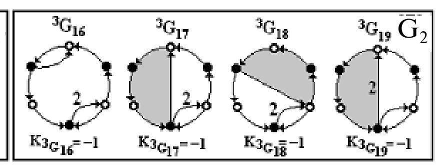
It can be shown that the graph composed of a cycle containing any number of positive pathways and any number of graphical elements (0)-loop in all cases is characterized by $K_{^kG_i} = -1$. Graphs $^3\boldsymbol{G}_9$, $^3\boldsymbol{G}_{10}$, and $^3\boldsymbol{G}_{11}$ contain two even-numbered cycles and one (0)-loop in different combinations. The following graphical rule can be suggested to characterize such structures: **any loop that is not incorporated in an even-numbered cycle reduces the numerical value of negative graph of order *k* by one (Rule 7).**

Two possible variants of negative graphs containing an autocatalytic loop ($^3\boldsymbol{G}_{13}$) and a product stoichiometric coefficient not equal to one ($^3\boldsymbol{G}_{12}$) are shown in scheme ($\mathbf{F_2}$) as an example.

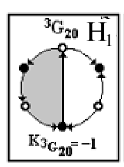


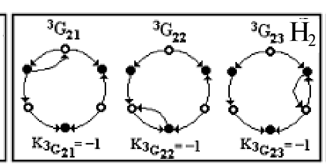
Schemes $\mathbf{G_1}$ and $\mathbf{G_2}$ show a group of the third order negative graphs containing one negative pathway. It should be emphasized that the necessary condition of the existence of such topological variants is the presence of a graphical element loop and/or the presence of at least one product stoichiometric coefficient larger than one.

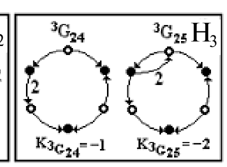




Schemes $\mathbf{H_1}$, $\mathbf{H_2}$, and $\mathbf{H_3}$ show a group of third order negative graphs containing two negative pathways. If each stoichiometric coefficient is equal to one and there is no elements loop, there is only one negative graph topology—$^3\boldsymbol{G}_{20}$.



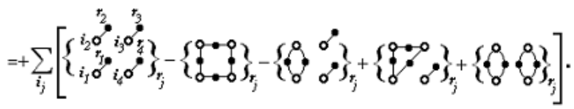




Graph $^3\boldsymbol{G}_{20}$ is a part of many biochemical systems. For example, it was found in complete graphs of many mechanisms of substrate inhibition in enzymatic reactions. Graphs $^3\boldsymbol{G}_{21}$, $^3\boldsymbol{G}_{22}$, and $^3\boldsymbol{G}_{23}$ represent all possible variants of topology of even-numbered cycle including two negative pathways and a graphical element (0)-loop. Scheme $\mathbf{H_3}$ shows two possible variants of negative graph topology, in which the weights (stoichiometric coefficients) of the graph pathways are larger than one ($^3\boldsymbol{G}_{24}$, $^3\boldsymbol{G}_{25}$).

4. Value and sign of coefficient a_4. Minimal negative graphs (oscillators) of fourth order. Coefficient $\boldsymbol{a_4}$ is the sum of all diagonal minors of the fourth order of the initial system Jacobian of order $\boldsymbol{m}$. By definition, the fourth order determinant is equal to the algebraic sum of 24 products of four elements of the determinant selected in accordance with a corresponding rule. Taking into consideration simple geometrical sense of each Jacobian element (table), it can be shown that coefficient $\boldsymbol{a_4}$ corresponds to an algebraic sum of four variants $g^{(I)}_{n_1}$, $g^{(II)}_{n_2}$, $g^{(III)}_{n_3}$ and $g^{(IV)}_{n_4}$ of subgraphs listed above:

$$a_4 = \sum_{n_1,n_2,n_3,n_4} (g^{(I)}_{n_1} + g^{(II)}_{n_2} + g^{(III)}_{n_3} + g^{(IV)}_{n_4}) = \qquad (30)$$

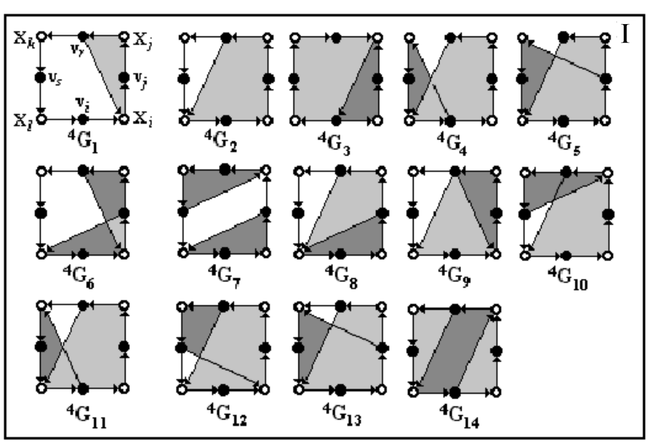


Let us compile a list of the fourth order negative graphs and basic graphical rules of their construction. In the simplest case the graphs contain no loops and all stoichiometric coefficients of reactions v_r, v_s, v_i, and v_j are equal to one each. In this case, the value of any fourth order negative graph is:

$$^4G_n = K_{^4G_n} \cdot \frac{v_r \cdot v_s \cdot v_i \cdot v_j}{x_i \cdot x_j \cdot x_k \cdot x_l}, \qquad (31)$$

where coefficient $K_{^4G_n} \geqslant -1$, and this value is determined by the number of even-numbered cycles rather than by the values of stoichiometric coefficients.

Scheme **I** shows a group of fourth order negative graphs, which contain only **positive pathways** if the degree of any substance node is equal to one.

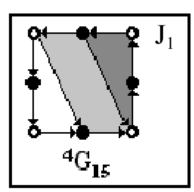


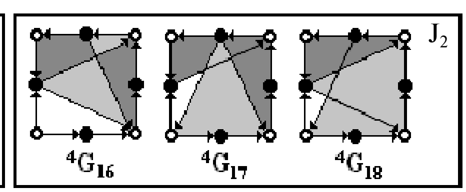
The graph in this case is negative if there are at least **two even-numbered cycles** with substances X_i, X_j, X_k, X_l capable of compensating positive contribution of single subgraph composed of half-pathway products. Graphs $^4\boldsymbol{G}_1$ and $^4\boldsymbol{G}_2$ contain two even-numbered cycles each. One of two even-numbered cycles includes the four substances, whereas the other cycle includes either two ($^4\boldsymbol{G}_1$) or three ($^4\boldsymbol{G}_2$) substances. Graphs $^4\boldsymbol{G}_3$, $^4\boldsymbol{G}_4$, and $^4\boldsymbol{G}_5$ do not contain cycles including the four substances. These graphs are formed by two even-numbered cycles including either two and three ($^4\boldsymbol{G}_3$, $^4\boldsymbol{G}_4$) substances or only three ($^4\boldsymbol{G}_5$) substances of the system. Graphs $^4\boldsymbol{G}_6$-$^4\boldsymbol{G}_{14}$ are derivatives of the graphs considered above and can be formed by three or more even-numbered cycles including four, three, and two substances. This example illustrates all variants of the fourth order graphs formed by six positive pathways containing three even-numbered cycles. Graphs ($^4\boldsymbol{G}_6$, $^4\boldsymbol{G}_7$), ($^4\boldsymbol{G}_8$-$^4\boldsymbol{G}_{11}$), and ($^4\boldsymbol{G}_{12}$-$^4\boldsymbol{G}_{14}$) represent combinations of even-numbered cycles with two, two-three, and three substances, respectively. The value of coefficient $K_{^4G_n}$ of negative graphs of this group containing two or more even-numbered cycles is:

$$K_{^4G_n} = -(N-1) \ ,$$

where $N \geq 2$ is the total number of even-numbered cycles in the fourth order negative graph.

Schemes $\mathbf{J_1}$ and $\mathbf{J_2}$ show a group of the fourth order negative graphs containing **one negative pathway**. In this case, one substance node of these graphs has the second degree, which gives rise to appearance of at least two positive subgraphs composed of products of half-pathways. Topological analysis allowed a simple graphical rule of construction of negative graphs of this group to be suggested. **The fourth order graph containing one negative pathway has a negative value only if it contains four even-numbered cycles formed by at least seven pathways, one of them being negative and the others positive (Rule 8).**

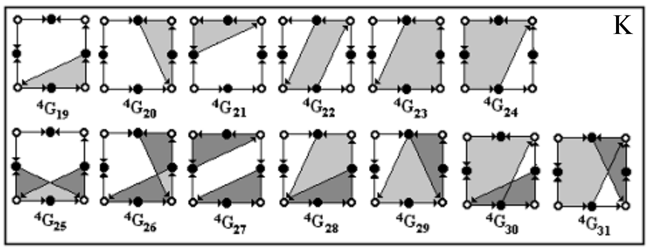




The topology of the graphs of this group falls into two variants. The graph of the first type, $^4\boldsymbol{G}_{15}$, contains one even-numbered cycle produced by positive pathways passing through the four substances, an even-numbered cycle produced by a negative pathway, and two even-numbered cycles passing through two and three substances, respectively. Graph $^4\boldsymbol{G}_{15}$ is the only negative graph of this type, unless the only one negative pathway is a part of the graph contour. The other combinations are either positive or equal to zero.

The contours of the graphs of the second type of this group (scheme $\mathbf{J_2}$) do not contain an even-numbered cycle passing through four substances. These graphs consist of one even-numbered cycle produced by a negative pathway and one of the following combinations of three even-numbered cycles: two-two-three and two-three-three substances (graphs $^4G_{16}$, $^4G_{17}$, and $^4G_{18}$, respectively). Graphs $^4G_{15}$-$^4G_{18}$ contain the minimum possible number of pathways. These negative graphs are characterized by the coefficient $K_{^4G_n} = -(N-1)$ in Eq. (31).

Scheme **K** shows the fourth order negative graphs containing two negative pathways, which are arranged as an even-numbered cycle passing through the four substances. The graphs of this group can be constructed using the following simple rule. **The fourth order graph containing two negative pathways, arranged as an even-numbered cycle containing four substances, is negative if and only if the graph also contains at least one even-numbered cycle produced by positive pathways (Rule 9).**



Scheme **K** shows all possible variants of topology of negative graphs containing two even-numbered cycles, one of which includes the four substances, whereas the other includes a cycle through two ($^4G_{19}$-$^4G_{22}$) or three ($^4G_{23}$, $^4G_{24}$) substances. Graphs $^4G_{19}$-$^4G_{24}$ are characterized by the coefficient $K_{^4G_n} = -(N-1)$ in Eq. (31).

The graphs characterized by the coefficient $K_{^4G_n} = -2$ in Eq. (31) are shown in scheme **K** as an example. All graphs $^4G_{25}$-$^4G_{31}$ are produced by the minimum possible number of pathways and include a combination of two even-numbered cycles composed of positive pathways through two and three substances. In addition, the negative graphs of this type differ from each other by the arrangement of negative pathways in the even-numbered cycle including the four substances.

Let us in conclusion list certain criteria inherent in the existence of a negative contribution (graph of order $\boldsymbol{k}$) to coefficient $\boldsymbol{a_k}$ ($\boldsymbol{k} = 1, 2, ..., \boldsymbol{m} - 1$) and a positive contribution to coefficient $\boldsymbol{a_m}$. The graph of order $\boldsymbol{k}$ is negative if the following conditions are met: **(i)** there are at least two even-numbered cycles passing through the same reaction nodes and substance nodes belonging to the same aggregate of substances; **(ii)** there is an even-numbered cycle, in which at least one variant of the pathway arrangement satisfies the condition $\beta_{ir} > 1$; **(iii)** there is at least one graphical element loop in an even-numbered cycle; and **(iiii)** there is at least one graphical element (+)-loop.

Coefficient $\boldsymbol{a_m}$ is positive if the following conditions are observed: **(j)** there is an odd-numbered cycle including all substances of the system; **(jj)** there are half-pathways that do not belong to any even-numbered cycle (e.g., reactions of matter exchange between the system and surrounding medium); **(jjj)** there are two or more only even-numbered or only odd-numbered cycles belonging to neither the same aggregate of substance nodes nor the same aggregate of reaction nodes.

The graphical rules considered above provide the opportunity to suggest a number of topological criteria for schemes of complex reactions, which indicate that the leading coefficient $\boldsymbol{a_m}$ is positive. For example, these criteria include so-called "dangling nodes" or "buffer stages". It was shown in [11-13] that incorporation of buffer stages into the kinetic models of the chemical reactions containing potential oscillators gives rise to generation of oscillations. Graphical analysis revealed that this was caused by elimination of the negative contribution to coefficient $\boldsymbol{a_m}$ associated with the negative subgraph, which determines itself a negative contribution to coefficient $\boldsymbol{a_k}$. In this case, the sufficient condition of existence of oscillations (10) is observed, the value of coefficient $\boldsymbol{a_m}$ is strictly negative, and the value of coefficient $\boldsymbol{a_k}$ ($\boldsymbol{k} = 1, 2, ..., \boldsymbol{m} - 1$) is alternating-sign.

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