DETECTING STRICTLY DETAILED BALANCED SUBNETWORKS IN OPEN CHEMICAL REACTION NETWORKS

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Abstract

The notion "strictly detailed balanced subnetwork" is introduced, for chemical reaction networks which are open and spatially homogeneous, to refer to any set of reactions the net rates of which vanish in each asymptotically stable steady state, regardless of the kinetic parameters of any reaction in the whole network. Necessary and sufficient conditions for sets of reactions to be strictly detailed balanced subnetworks are derived. An algorithm for detecting all reactions belonging to such subnetworks in systems of arbitrary stoichiometry is given, justified and applied to a realistic biochemical system. A computer program in PASCAL, performing the essential parts of this algorithm, is added.

1. Introduction

In 1902, Rudolf Wegscheider [1] drew attention to the fact that in some cases, mass action rate laws for chemical reactions as obtained from chemical kinetics are not consistent with the law of mass action as derived from thermodynamics, if the two following assumptions are made:

(1) the variations of molecule numbers are restricted only by stoichiometric conditions;

(2) the rate constants of all reactions are independent of each other.

As Lewis [2] proved in 1925 by using the principle of microreversibility, it is the second assumption which has to be dropped. Whereas Wegscheider himself considered rather special systems, the condition today bearing his name was later phrased for any cycle of monomolecular reactions with linear rate laws, in the form

$$\prod_j (k_j / k_{-j}) = 1, \tag{1.1}$$

(for example in [3,4]), where k_j and k_{-j} are the rate constants of forward and reverse reactions, respectively.

In a recent paper [5], we generalized condition (1.1) to systems of any stoichiometry and endowed with a generalized mass action kinetics (comprising usual mass action kinetics as a special case, provided that the reactions are reversible, cf. eq. (2.2) in the present paper). The generalized condition reads

$$\prod_j \tilde{q}_j^{\lambda_{jk}} = 1, \tag{1.2}$$

where $\tilde{q}_j$ is the apparent equilibrium constant of reaction R_j (defined as the equilibrium constant divided by the mass action ratio of the external species) and λ_{jk} are the elements of a matrix λ whose columns span the null space of the stoichiometric matrix $\boldsymbol{C}$. As for open reaction systems, condition (1.2) is a necessary and sufficient condition for the system to be detailed balanced. For the case of usual mass action kinetics, this assertion has been proved in a book by Volpert and Khudyayev ([7], pp. 366–372), of which we were informed only after publication of [5]. In closed systems, to which both Wegscheider [1] and Lewis [2] confined their considerations, condition (1.2) is always fulfilled.

To avoid confusion, we here emphasize the difference between the terms "detailed balanced state" and "detailed balanced reaction system". A state of a chemical system (characterized by a concentration vector) is detailed balanced if and only if all net reaction rates are zero, whereas a system is called detailed balanced if and only if all stationary states of this system are detailed balanced and at least one such state can be attained (cf. [6]).

Since open reaction systems are generally characterized by permanent exchange of matter, they are rather seldom in equilibrium. Yet, detailed balancing may in a stretched time scale be relevant to open networks if they are fast subsystems of greater networks, thus justifying the rapid-equilibrium approximation to be used [5,8].

Furthermore, detailed balancing can be observed in certain subsystems of open reaction systems regardless of the time scale chosen for the analysis. For motivation, let us consider a simplified reaction scheme of glycolysis in human red blood cells, as depicted in fig. 1. This scheme comprises the adenylate kinase reaction (R_6), the conversion of glucose-6-phosphate (G6P) into glucose-1-phosphate (G1P), which is catalyzed by phosphoglucomutase (R_5) (cf. [9]), and the reactions catalyzed by phosphofructokinase-2 (R_8) and fructose-2,6-bisphosphatase (R_4) (cf. [10]).

One can easily see that the phosphoglucomutase reaction (R_5) is a dead-end branch so that its net reaction rate, v_5, always equals zero whenever the considered network has attained a steady state. It is obvious that this feature is independent of the kinetic parameters of all the system's reactions.

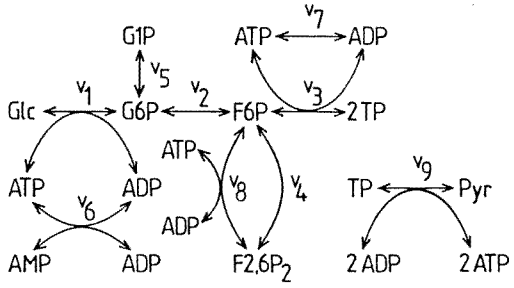

Fig. 1. Simplified reaction scheme of glycolysis inclusive of some adjacent reactions. Abbreviations: G6P: glucose-6-phosphate (X_1); F6P: fructose-6-phosphate (X_2); TP: pool of triose phosphates (X_3); G1P: glucose-1-phosphate (X_4); F2, $6P_2$: fructose-2,6-bisphosphate (X_5); ATP, ADP, and AMP have their usual meanings, their concentrations are denoted by X_6, X_7, and X_8, respectively. Glucose (Glc) and pyruvate (Pyr) are considered as external metabolites.

The situation changes when G1P and F6P are treated as external species, i.e. as reactants the concentrations of which are kept constant. Let us for a moment assume the kinetic functions to be linear, $v_1 = k_1 Glc - k_{-1} G6P$, $v_2 = k_2 G6P - k_{-2} F6P$, and $v_5 = k_5 G6P - k_{-5} G1P$, and the inequalities $(Glc\, q_1\, q_5)/G1P > 1$ and $(F6P\, q_5)/(G1P\, q_2) < 1$ to hold, where the italic symbols designate the concentrations of the corresponding reactants and $q_j = k_j/k_{-j}$ ($j = 1, 2, 5$). In this case, v_5 may be zero as well, namely, if

$$k_2 = k_1 \frac{Glc - G1P/(q_1 q_5)}{G1P/q_5 - F6P/q_2} . \tag{1.3}$$

However, this is, with respect to kinetic constants, a very special case.

To distinguish these two types of detailed balancing, in section 2 we define the notions "detailed balanced subnetwork" and " strictly detailed balanced subnetwork". The attribute "strict" is here used to refer to invariance upon alteration of the values of kinetic parameters.

It follows from the results of our previous paper [5] that if a reaction system is detailed balanced, then it has this property irrespective of its kinetic parameter values, since condition (1.2) contains only apparent equilibrium constants. Therefore, it is needless to use the notion "strict detailed balancing" for whole systems. For subsystems, however, this specification is of importance, as the example considered above shows. If G1P and F6P are external species and (1.3) is fulfilled, the subnetwork composed of reaction R_5 is detailed balanced but not strictly detailed balanced.

The motivation for dealing with strictly detailed balanced subnetworks is inferred from the following considerations. On modeling chemically reacting mixtures,

one usually begins with, or even restricts oneself to, the analysis of their stationary states (cf. [11–16]) and to the quasi-stationary states of fast subsystems (cf. [8,12,13]). This analysis is evidently simplified if all reactions which have, in steady state, a zero net reaction rate are detected at the very beginning.

Very frequently, simulation of reaction systems is hampered by incompleteness or unsatisfactory accuracy of the known data. Usually, thermodynamic parameters can be measured more accurately than kinetic ones (e.g. rate constants). In biochemical networks, fast reactions can, *in vivo*, generally be characterized only thermodynamically, i.e. in terms of equilibrium constants (cf. [12]). Obviously, this problem does not affect the steady-state modeling when reactions with unknown rate constants belong to a strictly detailed balanced subnetwork.

We believe that the new concept of strictly detailed balanced subnetworks will be of special relevance for biochemical reaction pathways, since in these systems kinetic parameters can vary more rapidly than in inanimate systems as, for instance, by regulation of enzyme activities.

Aiming at a description of regulatory properties of biochemical systems, Kacser and Burns [15], and Heinrich and Rapoport [16] developed, independently of each other, a formalism which is at present known as metabolic control analysis (see also [14]). In this analysis, the extent to which a steady-state flux v_j^s is controlled by a particular (enzymatic) reaction R_k is quantitatively expressed by the flux control coefficient C_k^j:

$$C_k^j = \frac{\partial \ln v_j^s}{\partial \ln p_k} \Big/ \frac{\partial \ln v_k}{\partial \ln p_k} . \tag{1.4}$$

Similarly, concentration control coefficients are defined. In (1.4), v_j^s is a function of system parameters only. In contrast, the rate v_k depends on concentrations of internal species as well as on parameters. p_k denotes any kinetic parameter (e.g. the concentration of the enzyme catalyzing reaction R_k) which enters only the rate equation of reaction R_k (cf. [14–16]).

C_k^j is not, however, defined when v_k and/or v_j^s are zero. In some cases, this discontinuity can be removed by defining $C_k^j = 0$. This is justified, at least whenever reaction R_j belongs to a strictly detailed balanced subnetwork, since no reaction can exert any control over the (zero) fluxes through such a subnetwork.

In complex systems, the strictly detailed balanced subnetworks often cannot be detected by inspection. An algorithm for this detection, which can also be performed by computer, would then be helpful. Such an algorithm, applicable to reaction networks with any stoichiometry and involving external species, is given in section 4 of the present paper. In section 5, this algorithm is applied to the biochemical system considered above. A computer program performing the essential steps of the algorithm is listed in the appendix. Section 2 is devoted to terminology. In section 3, we derive necessary and sufficient conditions for strict detailed balancing in subnetworks.

2. Notations and definitions

Throughout this paper, we use the symbols O and I, respectively, to denote the null matrix and the identity matrix of suitable dimension. Matrices $(\partial X_i / \partial Y_j)$ of partial derivatives are represented by the symbol X_Y, where X_i and Y_j are the elements of vectors X and Y, respectively.

The kernel (null-space) of a matrix A is denoted by $\ker(A)$. (diag X) stands for the diagonal matrix containing the components of vector X as diagonal elements. For basic notions (rank, base, kernel, etc.) and relations of linear algebra, the reader is referred to [17–20].

Consider a set $\mathcal{R}$ of r chemical reactions, $\mathcal{R} = \{R_1, R_2, \ldots, R_r\}$, which interconvert $n + m$ reactants with m of these being external species. Under the assumption of spatial homogeneity of concentrations, the temporal evolution of the system can be described by the O.D.E. system

$$\frac{dX}{dt} = C\,V(X), \tag{2.1}$$

where X, V, and C denote the vector of concentrations X_i of internal species, the vector of net reaction rates v_j, and the stoichiometric matrix with elements c_{ij}, respectively (cf. [11]).

In our previous paper [5], we introduced a generalized mass action kinetics,

$$v_j(X) = G_j(X)\,\Gamma_j\left[-\ln\left(\prod_{i=1}^{n} X_i^{c_{ij}}/\tilde{q}_j\right)\right], \qquad j = 1, \ldots, r, \tag{2.2}$$

which is a convenient generalization of a large multitude of rate laws. The expression in square brackets in (2.2), which will be abbreviated to A_j, is proportional to the affinity of reaction R_j. $\tilde{q}_j$ is an apparent equilibrium constant. $G_j(X)$ is, for every j, assumed to be positive for any X with positive components. The $\Gamma_j(A_j)$ are strictly monotonic increasing functions passing through the origin of coordinates.

By now, we have realized that expression (2.2) may be simplified to

$$v_j(X) = \bar{G}_j(X)\,A_j(X) \tag{2.3}$$

by the definition

$$\bar{G}_j(X) = G_j(X)\,\Gamma_j[A_j(X)]/A_j(X). \tag{2.4}$$

Since $\Gamma_j(A_j)$ has always the same sign as A_j, $\bar{G}_j(X)$ is positive whenever $G_j(X)$ is positive.

Nevertheless, function (2.2) is a convenient expression, at least for (enzyme-) catalyzed reactions inasmuch as $\Gamma_j(A_j)$ may express the catalytic power and $G_j(X)$ the

inhibitory, activatory, competitive, and other modifying properties. In the present paper, we employ expression (2.3) rather than (2.2) in order to simplify the mathematical presentation.

For the purpose of our paper, it is appropriate to define the term "reaction network" as a reaction system which is specified thermodynamically and whose kinetic properties (i.e. the functions $G_j(\boldsymbol{X})$ and the values of kinetic parameters) are assumed to be specified in each concrete situation, but not necessarily known. Moreover, the values of kinetic parameters are allowed to change with time. This can be concisely stated as follows.

DEFINITION 1

A *chemical reaction network* is a set of reaction stoichiometries $\mathcal{R} = \{R_1, \ldots, R_r\}$ endowed with generalized mass action kinetics with specified apparent equilibrium constants $\tilde{q}_j$, $j = 1, \ldots, r$.

Remark

This definition is a slightly modified variant of the terminology of Clarke, who defines a chemical system to be a set of reaction stoichiometries endowed with rate laws with specified parameter values, and a chemical network to be a set of chemical systems, one system for each value of a parameter vector which lies in a specified domain of parameter space ([11], p. 8).

DEFINITION 2

A *chemical subnetwork* of a given reaction network with reaction set $\mathcal{R}$ is a subset of $\mathcal{R}$, $\mathcal{R}^{(i)} \subset \mathcal{R}$, where each reaction R_j from $\mathcal{R}^{(i)}$ has the same apparent equilibrium constant as it has in the whole network.

DEFINITION 3

A chemical subnetwork (with $\mathcal{R}^{(i)}$ being its subset of reactions) will be called *detailed balanced* if and only if the net reaction rates v_j for all j with $R_j \in \mathcal{R}^{(i)}$ vanish in every asymptotically stable steady state of the whole network.

Remark

The notion of asymptotic stability will, in the present paper, be interpreted in the sense that rank($\boldsymbol{C}$) eigenvalues of the Jacobian matrix of (2.1) have negative real parts in the considered steady states. In the case rank($\boldsymbol{C}$) < n, we need not take all n eigenvalues into consideration because n – rank($\boldsymbol{C}$) of them always vanish due to conservation relations (cf. [11,14]).

DEFINITION 4

A subnetwork of a given reaction network will be called *strictly detailed balanced* if and only if it is detailed balanced for any parameter value set, the apparent equilibrium constants $\tilde{q}_j$, $j = 1, \ldots, r$, being fixed and condition $G_j(X) > 0$ being fulfilled for any j.

Remark

For the calculations in the following section, it is of importance that the term "parameter" used in definition 4 applies to any quantity (apart from the stoichiometric coefficients and the apparent equilibrium constants) influencing the reaction rates of this subnetwork, even if this quantity does not enter the rate law. For example, many rate laws, for the sake of simplicity, are linear functions. In these cases, the complete nonlinear rate law has to be considered in definition 4. Moreover, it is of no relevance whether some considered parameter can actually be changed in the real system.

3. A generalized Wegscheider condition for subnetworks

We now investigate the question under which conditions a chemical subnetwork is strictly detailed balanced. Let

$$\gamma = \operatorname{rank}(\boldsymbol{C}). \tag{3.1}$$

Since in the case $\gamma = r$ the whole network attains a globally stable equilibrium (cf. [5]), we confine the analysis to the case $\gamma < r$. Let λ be an $r \times (r - \gamma)$ matrix whose columns are a base of $\ker(\boldsymbol{C})$:

$$\boldsymbol{C}\lambda = \boldsymbol{O}. \tag{3.2}$$

Matrix λ, which will be called a null-space matrix, is not uniquely determined. It can be transformed by the following two operations to give another matrix $\hat{\lambda}$ whose columns span the null-space of $\boldsymbol{C}$ as well.

(O1) Multiplication by a non-singular $(r - \gamma) \times (r - \gamma)$ matrix $\boldsymbol{Q}$ from the right:

$$\hat{\Lambda} = \Lambda \boldsymbol{Q}. \tag{3.3}$$

(O2) Permutation of rows, i.e. multiplication by a permutation matrix $\boldsymbol{P}$ from the left:

$$\hat{\Lambda} = \boldsymbol{P}\Lambda. \tag{3.4}$$

Operation (O2) entails a renumbering of columns of $\boldsymbol{C}$.

The algorithm for detecting all reactions belonging to a strictly detailed balanced subnetwork will be based on the following:

THEOREM

A subnetwork of a given reaction network is strictly detailed balanced if and only if the following two conditions are fulfilled:

(C1) The null-space matrix λ can be transformed by operations (O1) and (O2) into a generalized diagonal matrix

$$\lambda = \begin{pmatrix} \lambda^{(1)} & O \\ O & \lambda^{(2)} \end{pmatrix}, \tag{3.5}$$

where $\lambda^{(1)}$ and $\lambda^{(2)}$ are a $g \times l$ matrix and an $(r - g) \times (r - \gamma - l)$ matrix, respectively, with the rows of the submatrix $(\lambda^{(1)}\ O)$ corresponding to those columns in C which belong to the considered subnetwork. In (3.5), the cases $l = 0$ and $l = r - \gamma$ are admitted as well, that is, the number of columns of $\lambda^{(1)}$ or of $\lambda^{(2)}$ may be zero.

(C2) Either the number of columns of $\lambda^{(1)}$, l, is zero, or the equation

$$\lambda^{(1)^{\mathrm{T}}} \ln \tilde{q}^{(1)} = 0 \tag{3.6}$$

is fulfilled, where $\tilde{q}^{(1)}$ is, after the renumbering of reactions according to operation (O2), that subvector of $\tilde{q}$ which corresponds to $\lambda^{(1)}$, i.e.

$$\tilde{q} = \begin{pmatrix} \tilde{q}_1 \\ \tilde{q}_2 \end{pmatrix}, \quad \dim(\tilde{q}_1) = g. \tag{3.7a,b}$$

Proof

To prove the sufficiency, we assume conditions (C1) and (C2) to hold true. In accordance with (3.7), we partition C, V, and $\bar{G}(X) = (G_1(X), \ldots, G_r(X))^{\mathrm{T}}$ as

$$C = \left(C^{(1)}\ C^{(2)}\right), \quad V = \begin{pmatrix} V^{(1)} \\ V^{(2)} \end{pmatrix}, \quad \bar{G}(X) = \begin{pmatrix} \bar{G}^{(1)} \\ \bar{G}^{(2)} \end{pmatrix}. \tag{3.8a,b,c}$$

Equations (3.2), (3.5), and (3.8a) imply

$$C^{(1)}\lambda^{(1)} = 0. \tag{3.9}$$

From (3.9) and the steady-state equation $C^{(1)}V^{(1)} = 0$, it follows that $V^{(1)}$ can be written as

$$\boldsymbol{V}^{(1)} = \lambda^{(1)}\boldsymbol{Z}, \tag{3.10}$$

where $\boldsymbol{Z}$ is some l-vector.

Using (2.3), we can write the function $\boldsymbol{V}^{(1)}(\boldsymbol{X})$ as

$$\boldsymbol{V}^{(1)}(\boldsymbol{X}) = \left(\operatorname{diag} \overline{\boldsymbol{G}}^{(1)}(\boldsymbol{X})\right)\left(\ln \tilde{\boldsymbol{q}}^{(1)} - \boldsymbol{C}^{(1)^{\mathrm{T}}} \ln \boldsymbol{X}\right). \tag{3.11}$$

Multiplication of eq. (3.11) by $\boldsymbol{V}^{(1)^{\mathrm{T}}}(\operatorname{diag} 1/\overline{\boldsymbol{G}}^{(1)})$ from the left yields

$$\boldsymbol{V}^{(1)^{\mathrm{T}}}\left(\operatorname{diag} 1/\overline{\boldsymbol{G}}^{(1)}\right)\boldsymbol{V}^{(1)} = \boldsymbol{V}^{(1)^{\mathrm{T}}} \ln \tilde{\boldsymbol{q}}^{(1)} - \boldsymbol{V}^{(1)^{\mathrm{T}}}\boldsymbol{C}^{(1)^{\mathrm{T}}} \ln \boldsymbol{X}. \tag{3.12}$$

This equation can, by virtue of (3.6), (3.9), and (3.10), be simplified to

$$\boldsymbol{V}^{(1)^{\mathrm{T}}}\left(\operatorname{diag} 1/\overline{\boldsymbol{G}}^{(1)}\right)\boldsymbol{V}^{(1)} = 0. \tag{3.13}$$

Since all $\overline{G}_j$ are positive, the quadratic form on the l.h.s. of (3.13) is positive definite. Consequently, eq. (3.13) holds if and only if all components of $\boldsymbol{V}^{(1)}$ vanish, which completes the proof of the "if" part of the theorem.

In order to prove the necessity, we consider the subnetwork corresponding to $\boldsymbol{V}^{(1)}$ to be strictly detailed balanced. Then we have

$$\boldsymbol{V}^{(1)} = 0, \tag{3.14}$$

which implies, due to (2.3) and $G_j(\boldsymbol{X}) > 0$ for all j,

$$\ln \tilde{\boldsymbol{q}}^{(1)} - \boldsymbol{C}^{(1)^{\mathrm{T}}} \ln \boldsymbol{X} = 0. \tag{3.15}$$

According to definition 4 in section 2, eq. (3.15) has to hold regardless of the values of kinetic parameters (which will be gathered in a vector $\boldsymbol{p}$). Thus, we have to invoke that the first differential of the l.h.s. in (3.15) (denoted by $\mathrm{d}\boldsymbol{A}$) with any increment $\mathrm{d}\boldsymbol{p}$ equals the null vector:

$$\mathrm{d}\boldsymbol{A}(\boldsymbol{p}, \mathrm{d}\boldsymbol{p}) = \mathrm{d}\left(\ln \tilde{\boldsymbol{q}}^{(1)^{\mathrm{T}}} - \boldsymbol{C}^{(1)^{\mathrm{T}}} \ln \boldsymbol{X}\right) = 0. \tag{3.16}$$

This equation leads to

$$\boldsymbol{C}^{(1)^{\mathrm{T}}}(\operatorname{diag} 1/\boldsymbol{X})\,\mathrm{d}\boldsymbol{X} = 0. \tag{3.17}$$

We now have to take into consideration that in the case of $\gamma < n$, there exists $n - \gamma$ invariants involving reactant concentrations,

$$\boldsymbol{B}\boldsymbol{X}(t) = \boldsymbol{K}, \tag{3.18}$$

where $\boldsymbol{K}$ denotes a vector of $n - \gamma$ constants and $\boldsymbol{B}$ is an $(n - \gamma) \times n$ matrix defined by

$$\boldsymbol{B}\boldsymbol{C} = 0 \tag{3.19}$$

(cf. [13]). Let the rows of $\boldsymbol{C}$ be rearranged so that the upper γ rows (which form the matrix $\overline{\boldsymbol{C}}$) are linearly independent:

$$\boldsymbol{C} = \begin{pmatrix} \overline{\boldsymbol{C}} \\ \hat{\boldsymbol{C}} \end{pmatrix}. \tag{3.20}$$

We partition $\boldsymbol{C}^{(1)}$ and $\boldsymbol{C}^{(2)}$ in accordance with (3.20) as

$$\boldsymbol{C}^{(1)} = \begin{pmatrix} \overline{\boldsymbol{C}}^{(1)} \\ \hat{\boldsymbol{C}}^{(1)} \end{pmatrix}, \quad \boldsymbol{C}^{(2)} = \begin{pmatrix} \overline{\boldsymbol{C}}^{(2)} \\ \hat{\boldsymbol{C}}^{(2)} \end{pmatrix}. \tag{3.21}$$

This rearrangement given, $\boldsymbol{B}$ may be chosen as

$$\boldsymbol{B} = (\overline{\boldsymbol{B}}\ \boldsymbol{I}), \tag{3.22}$$

with $\overline{\boldsymbol{B}}$ being an $(n - \gamma) \times \gamma$ matrix, since the rows of $\hat{\boldsymbol{C}}$ must be linear combinations of the rows of $\overline{\boldsymbol{C}}$, that is,

$$\hat{\boldsymbol{C}} = -\overline{\boldsymbol{B}}\,\overline{\boldsymbol{C}}. \tag{3.23}$$

Partitioning $\boldsymbol{X}$ corresponding to (3.20) as

$$\boldsymbol{X} = \begin{pmatrix} \overline{\boldsymbol{X}} \\ \hat{\boldsymbol{X}} \end{pmatrix}, \quad \dim(\overline{\boldsymbol{X}}) = \gamma, \tag{3.24a,b}$$

we may write $\hat{\boldsymbol{X}}$ in terms of $\overline{\boldsymbol{X}}$ as follows:

$$\hat{\boldsymbol{X}} = \boldsymbol{K} - \overline{\boldsymbol{B}}\,\overline{\boldsymbol{X}}. \tag{3.25}$$

Using (3.23), (3.24a), and (3.25), we may rewrite (3.17) as

$$\overline{C}^{(1)^{\mathrm{T}}} M \, \mathrm{d}\overline{X} = 0, \tag{3.26}$$

with

$$M = \begin{pmatrix} I \\ -\overline{B} \end{pmatrix}^{\mathrm{T}} \left(\operatorname{diag} \frac{1}{X} \right) \begin{pmatrix} I \\ -\overline{B} \end{pmatrix}. \tag{3.27}$$

The increments $\mathrm{d}p$ and $\mathrm{d}\overline{X}$ are not independent since the system is to continue subsisting in steady state. Therefore, the total differential of the r.h.s. in (2.1) must equal the null vector:

$$C \, \mathrm{d}V \left[\overline{X}, \, \mathrm{d}\overline{X}(p, \mathrm{d}p), \, p, \, \mathrm{d}p \right] = O. \tag{3.28}$$

Using algebraic rules for differentials (cf. [19], ch. 5), we obtain

$$C V_{\overline{X}} \, \mathrm{d}\overline{X}(p, \mathrm{d}p) + C V_p \, \mathrm{d}p = O. \tag{3.29}$$

Since the rows of $\hat{C}$ are linearly dependent on the rows of $\overline{C}$ (cf. decomposition (3.20)), eq. (3.29) is equivalent to

$$\overline{C} V_X X_{\overline{X}} \, \mathrm{d}\overline{X}(p, \mathrm{d}p) + \overline{C} V_p \, \mathrm{d}p = O, \tag{3.30}$$

where the chain rule of differentiation has been used. The matrix

$$J = \overline{C} V_X X_{\overline{X}} \tag{3.31}$$

can be considered as a reduced Jacobian matrix of the O.D.E. system (2.1), a Jacobian which has been "rid" of the vanishing eigenvalues. It can be shown that J is non-singular and, thus, invertible whenever γ eigenvalues of the complete Jacobian CV_X of the O.D.E. system (2.1) have negative real parts [11,14]. Since we used this very assumption above (cf. the remark to definition 3), we may transform eq. (3.30) to

$$\mathrm{d}\overline{X} = -J^{-1} \overline{C} V_p \, \mathrm{d}p. \tag{3.32}$$

Substituting eq. (3.32) into (3.26), we obtain

$$\overline{C}^{(1)^{\mathrm{T}}} M J^{-1} \overline{C} V_p = O, \tag{3.33}$$

since (3.32) is to hold for arbitrary parameter changes $\mathrm{d}p$ being sufficiently small so as not to cause the steady state to become unstable. The matrix V_p can be obtained from (2.3):

$$V_p = \left[\operatorname{diag}(\ln \tilde{q} - C^{\mathrm{T}} \ln X)\right] \overline{G}_p . \tag{3.34}$$

In (3.34), the fact that the p_k are kinetic parameters rather than equilibrium constants and that they, accordingly, enter the functions $\overline{G}_j(X)$ rather than the affinities $A_j(X)$ has been taken into account. For this reason and due to eq. (3.15), we have

$$V_p^{(1)} = O , \tag{3.35}$$

which, upon substitution into (3.33), gives

$$\overline{C}^{(1)^{\mathrm{T}}} M J^{-1} \overline{C}^{(2)} V_p^{(2)} = 0 . \tag{3.36}$$

We now consider such a family p of external parameters which is rich enough for the matrix $V_p^{(2)}$ to have full rank $r - g$. Such a set p can always be chosen, since arbitrary parameter changes are allowed (see also the remark to definition 4 and considerations in [14], sect. 3B). We can then write

$$\operatorname{rank}(\overline{C}^{(2)} V_p^{(2)}) = \operatorname{rank}(\overline{C}^{(2)}) . \tag{3.37}$$

Equation (3.36) implies that the column vectors of the matrix $MJ^{-1}\overline{C}^{(2)}V_p^{(2)}$ have to be contained in the kernel of $\overline{C}^{(1)^{\mathrm{T}}}$, whence we can conclude that

$$\operatorname{rank}(M J^{-1} \overline{C}^{(2)} V_p^{(2)}) \leq \dim[\ker(\overline{C}^{(1)})] = \gamma - \operatorname{rank}(\overline{C}^{(1)}) . \tag{3.38}$$

Since M has a special symmetric structure (cf. eq. (3.27)), it is, due to a theorem of matrix algebra (theorem 5 in [20], p. 129), positive definite and, hence, non-singular. Therefore, also MJ^{-1} is non-singular. Consequently, inequality (3.38) implies

$$\operatorname{rank}(\overline{C}^{(2)} V_p^{(2)}) \leq \gamma - \operatorname{rank}(C^{(1)}) . \tag{3.39}$$

Equations (3.37) and (3.39) give

$$\gamma \geq \operatorname{rank}(\overline{C}^{(1)}) + \operatorname{rank}(\overline{C}^{(2)}) . \tag{3.40}$$

Since the rows of $\hat{C}^{(1)}$ and $\hat{C}^{(2)}$ are linearly dependent on the rows of $\overline{C}^{(1)}$ and $\overline{C}^{(2)}$, respectively, inequality (3.40) is equivalent to

$$\gamma \geq \operatorname{rank}(C^{(1)}) + \operatorname{rank}(C^{(2)}) . \tag{3.41}$$

Due to the decomposition of C given by (3.8a), (3.41) leads to

$$\gamma = \text{rank}\,(\boldsymbol{C}^{(1)}) + \text{rank}\,(\boldsymbol{C}^{(2)}). \tag{3.42}$$

From (3.42), we may derive

$$\dim[\ker(\boldsymbol{C}^{(1)})] + \dim[\ker(\boldsymbol{C}^{(2)})] = r - \gamma. \tag{3.43}$$

Since $r - \gamma$ is the dimension of $\ker(\boldsymbol{C})$, (3.8a) and (3.43) give

$$\ker(\boldsymbol{C}) = \ker(\boldsymbol{C}^{(1)}) \oplus \ker(\boldsymbol{C}^{(2)}), \tag{3.44}$$

where $\oplus$ refers to the direct sum.

In the same way as we defined matrix λ as the null-space matrix associated with the stoichiometric matrix $\boldsymbol{C}$ (cf. eq. (3.2)), we now define, for the submatrices $\boldsymbol{C}^{(1)}$ and $\boldsymbol{C}^{(2)}$, the null-space matrices $\lambda^{(1)}$ and $\lambda^{(2)}$, respectively. By virtue of this definition, we can write

$$(\boldsymbol{C}^{(1)}\ \boldsymbol{C}^{(2)}) \begin{pmatrix} \lambda^{(1)} & \boldsymbol{O} \\ \boldsymbol{O} & \lambda^{(2)} \end{pmatrix} = \boldsymbol{O}. \tag{3.45}$$

Equation (3.44) implies that the generalized diagonal matrix on the l.h.s. of (3.45) has $r - \gamma$ columns. All of these columns are linearly independent, since so are the columns of $\lambda^{(1)}$ and $\lambda^{(2)}$. Consequently, the generalized diagonal matrix on the l.h.s. of (3.45) is an admissible null-space matrix λ, so that condition (C1) follows from the property of the subnetwork corresponding to $\boldsymbol{V}^{(1)}$ to be strictly detailed balanced. If $l > 0$, condition (C2) is, due to (3.45), easily obtained by multiplying eq. (3.15) by $\lambda^{(1)^{\mathrm{T}}}$ from the left. If $l = 0$, that is, if (3.5) simplifies to

$$\lambda = \begin{pmatrix} \boldsymbol{O} \\ \lambda^{(2)} \end{pmatrix}, \tag{3.46}$$

all reactions of the considered subnetwork are, in any steady state, detailed balanced since the corresponding rows in λ contain zeroes only. This completes the proof of the theorem.

If the whole reaction network is to be examined as to whether or not it is strictly detailed balanced, (3.5) "degenerates" to

$$\lambda = \lambda^{(1)}, \tag{3.47}$$

so that condition (C2) coincides with the generalized Wegscheider condition as derived in [5], as should be expected. Accordingly, conditions (C1) and (C2) can be regarded as a generalized Wegscheider condition for subnetworks.

4. The algorithm

Using the results of the previous section, the following algorithm for detecting the largest detailed balanced subnetwork can be derived. The basic idea is to find a canonical form of matrix λ with respect to the group of transformations (O1) and (O2), which is a subgroup of the set of all equivalence transformations of $r \times r$ matrices (cf. [18], ch. 17).

We first give the algorithm and, thereafter, its justification.

(1) Transform matrix C by the Gaussian elimination method (given, for example, in [17], ch. 2) to an echelon form, C',

$$C' = \begin{pmatrix} c'_{11} & c'_{12} & \cdots & & & c'_{1r} \\ 0 & c'_{22} & & & & \vdots \\ 0 & 0 & \ddots & & & \vdots \\ \vdots & \vdots & & c'_{\gamma\gamma} & c'_{\gamma,\gamma+1} \cdots & c'_{\gamma r} \\ 0 & 0 & & & 0 \cdots & 0 \\ \vdots & \vdots & & & \vdots & \vdots \\ 0 & 0 & & & 0 \cdots & 0 \end{pmatrix}. \tag{4.1}$$

Calculate a set of basic solutions of the homogeneous equation system

$$C'\lambda_{\cdot k} = O \tag{4.2}$$

as usual in the Gaussian elimination method, i.e. by putting

$$\lambda_{ik} = \delta_{i-\gamma,k}, \quad i = \gamma+1, \ldots, r;\ k = 1, \ldots, r-\gamma \tag{4.3}$$

and by calculating the other λ_{ik} in a recursive way,

$$\lambda_{ik} = \left(-c'_{i,\gamma+k} - \sum_{m=i+1}^{\gamma} c'_{im}\lambda_{mk}\right) \Big/ c'_{ii}. \tag{4.4}$$

(2) All reactions which correspond to rows in λ that contain only zeroes belong to a strictly detailed balanced subnetwork. For the further analysis, cancel these rows (say, f in number) in λ. Let the resulting matrix be denoted by Λ.

(3) Rearrange the rows and columns of Λ in such a way that it forms a generalized diagonal matrix

$$\Lambda = \begin{pmatrix} \Lambda^{(1)} & & & \\ & \Lambda^{(2)} & & \boldsymbol{O} \\ & & \ddots & \\ & \boldsymbol{O} & & \Lambda^{(\mu)} \end{pmatrix}, \tag{4.5}$$

where none of the submatrices $\Lambda^{(k)}$ represents a generalized diagonal matrix in any rearrangement of rows and columns of $\Lambda^{(k)}$ whatsoever. Denote the numbers of rows and columns of $\Lambda^{(k)}$ by r_k and l_k, respectively. Then we have

$$\sum_{k=1}^{\mu} r_k = r - f, \quad \sum_{k=1}^{\mu} l_k = r - \gamma. \tag{4.6a,b}$$

(4) Decompose the vector $\ln \tilde{q}$ according to

$$\ln \tilde{q}^{\mathrm{T}} = \left(\ln \tilde{q}^{(1)^{\mathrm{T}}}, \ldots, \ln \tilde{q}^{(\mu)^{\mathrm{T}}}\right), \tag{4.7}$$

with $\ln \tilde{q}^{(k)}$ having r_k components. Check for each $k = 1, \ldots, \mu$ whether

$$\Lambda^{(k)^{\mathrm{T}}} \ln \tilde{q}^{(k)} = \boldsymbol{O} \tag{4.8}$$

is fulfilled. If it is for some k, the corresponding subnetwork is a strictly detailed balanced subnetwork. All of these subnetworks together may be looked upon as one compound network, which obviously also fulfills conditions (C1) and (C2). This subnetwork, together with the detailed balanced reactions detected in step (2), forms the maximum strictly detailed balanced subnetwork.

Justification of the algorithm

It follows from the theory underlying the Gaussian elimination method that the equation system (4.2) has the same manifolds of solutions as the equation system $\boldsymbol{C}\lambda_{\cdot k} = 0$. Therefore, the solutions $\lambda_{\cdot k}$ as calculated in step (1) form an admissible null-space matrix, which can be partitioned as

$$\lambda = \begin{pmatrix} \hat{\lambda} \\ \boldsymbol{I} \end{pmatrix}. \tag{4.9}$$

The detection of a strictly detailed balanced subnetwork in step (2) is a straightforward application of conditions (C1) and (C2) for the case $l = 0$.

In section 3, we considered a bipartite decomposition of the whole network and, accordingly, a decomposition of λ into two diagonal blocks. However, for some systems, λ can be partitioned into more than two blocks. Since we do not know from the

beginning which of these submatrices fulfills eq. (3.6), we cannot immediately decide which bipartite decomposition of λ is to be used. Therefore, in step (3) we determine a representation of λ with the maximum number of diagonal blocks possible, i.e. a canonical form as alluded to at the beginning of section 4. According to (4.9), Λ can be partitioned as

$$\Lambda = \begin{pmatrix} \hat{\Lambda} \\ \boldsymbol{I} \end{pmatrix}. \tag{4.10}$$

To justify step (3), we have to show that Λ as given by (4.10) can actually be rearranged so that it fulfills eq. (4.5), with μ being the maximum possible number of diagonal blocks. To this end, we cancel all columns in $\boldsymbol{C}$ which correspond to zero rows in λ. Let the resulting matrix be denoted by $\boldsymbol{D}$. We then have

$$\boldsymbol{D}\,\Lambda = \boldsymbol{O}. \tag{4.11}$$

Let Λ^{I} be the representation of Λ as constructed by the algorithm, i.e. as given by (4.10), and let $\boldsymbol{D}^{\mathrm{I}}$ be that representation of $\boldsymbol{D}$ which corresponds, in its arrangement of columns, to Λ^{I}. Let Λ^{II} denote a matrix that fulfills eq. (4.5), with μ having the property mentioned above. However, Λ^{II} need not necessarily result from Λ^{I} by interchange of rows and columns. Let $\boldsymbol{D}^{\mathrm{II}}$ be that form of $\boldsymbol{D}$ which corresponds, in its arrangement of columns, to Λ^{II}. We partition $\boldsymbol{D}^{\mathrm{II}}$ in accordance with (4.5):

$$\boldsymbol{D}^{\mathrm{II}} = \left(\boldsymbol{D}^{(1)} \quad \boldsymbol{D}^{(2)} \quad \ldots \quad \boldsymbol{D}^{(\mu)}\right). \tag{4.12}$$

$\boldsymbol{D}^{(k)}$ then has r_k columns. From eqs. (4.5), (4.11), and (4.12), we obtain

$$\boldsymbol{D}^{(k)}\Lambda^{(k)} = \boldsymbol{O}, \quad k = 1, \ldots, \mu. \tag{4.13}$$

Since Λ^{I} has the special structure given by (4.10) and $\hat{\Lambda}$ is a $(\gamma - f) \times (r - \gamma)$ matrix, $\boldsymbol{D}^{\mathrm{I}}$ can be partitioned as

$$\boldsymbol{D}^{\mathrm{I}} = \left(\boldsymbol{D}_1^{\mathrm{I}} \quad \boldsymbol{D}_2^{\mathrm{I}}\right), \tag{4.14}$$

with $\boldsymbol{D}_1^{\mathrm{I}}$ being an $n \times (\gamma - f)$ matrix of full rank.

In each submatrix $\boldsymbol{D}^{(k)}$, there are $r_k - l_k$ columns which are also columns of $\boldsymbol{D}_1^{\mathrm{I}}$. If there were more than $r_k - l_k$ such columns, which are linearly independent of each other since so are the columns of $\boldsymbol{D}_1^{\mathrm{I}}$, eq. (4.13) could not be fulfilled with $\Lambda^{(k)}$ having rank l_k. If there were less than $r_k - l_k$ such columns, the equation

$$\sum_{k=1}^{\mu} (r_k - l_k) = \gamma - f,$$

which follows from (4.6a,b), would imply that another submatrix $\boldsymbol{D}^{(j)}$ would contain more than $r_j - l_j$ columns which coincide with columns of $\boldsymbol{D}_1^{\mathrm{I}}$. From these considerations, it also follows that any $\boldsymbol{D}^{(k)}$ has rank $r_k - l_k$.

Now, we interchange the columns of $\boldsymbol{D}^{\mathrm{II}}$ so that all the $\gamma - f$ linearly independent columns defined above enter each submatrix $\boldsymbol{D}^{(k)}$ on the left-hand side of this submatrix. Then, each $\boldsymbol{D}^{(k)}$ can be partitioned as

$$\boldsymbol{D}^{(k)} = \left(\boldsymbol{D}_1^{(k)} \quad \boldsymbol{D}_2^{(k)}\right), \tag{4.15}$$

with $\boldsymbol{D}_1^{(k)}$ being an $n \times (r_k - l_k)$ matrix with rank $r_k - l_k$. Since the columns of $\boldsymbol{D}_2^{(k)}$ are linearly dependent on the columns of $\boldsymbol{D}_1^{(k)}$, there must exist matrices $\boldsymbol{T}^{(k)}$ $(k = 1, \ldots, \mu)$ with

$$\boldsymbol{D}_2^{(k)} = \boldsymbol{D}_1^{(k)} \boldsymbol{T}^{(k)}. \tag{4.16}$$

From (4.16), we can conclude that the $r_k \times l_k$ matrix

$$\Lambda^{\mathrm{III}(k)} = \begin{pmatrix} -\boldsymbol{T}^{(k)} \\ \boldsymbol{I} \end{pmatrix} \tag{4.17}$$

is an admissible matrix $\Lambda^{(k)}$ since it fulfills eq. (4.13). It remains to show that Λ^{III}, which is defined by (4.5) with $\Lambda^{(k)}$ given by (4.17), can be obtained from Λ^{I} by permuting rows and columns. To this end, we sort the rows of Λ^{III} so that all rows which correspond to a column of a submatrix $\boldsymbol{D}_2^{(k)}$, i.e. which represent a row of an identity matrix, get to the bottom of Λ:

$$\Lambda^{\mathrm{IV}} = \begin{pmatrix} \Lambda_1^{\mathrm{IV}} \\ \boldsymbol{I} \end{pmatrix}. \tag{4.18}$$

The matrices Λ^{I} and Λ^{IV} must be transformable into each other by the operations (O1) and (O2):

$$\Lambda^{\mathrm{IV}} = \boldsymbol{P} \Lambda^{\mathrm{I}} \boldsymbol{Q}. \tag{4.19}$$

After the permutation leading to (4.18), all columns of submatrices $\boldsymbol{D}_2^{(k)}$ form an $n \times (r - \gamma)$ matrix $\boldsymbol{D}_2^{\mathrm{IV}}$ on the right-hand side of $\boldsymbol{D}$:

$$\boldsymbol{D}^{\mathrm{IV}} = \left(\boldsymbol{D}_1^{\mathrm{IV}} \quad \boldsymbol{D}_2^{\mathrm{IV}}\right). \tag{4.20}$$

Since all columns of any submatrix $\boldsymbol{D}_1^{(k)}$ coincide with columns of $\boldsymbol{D}_1^{\mathrm{I}}$, the submatrices $\boldsymbol{D}_1^{\mathrm{IV}}$ and $\boldsymbol{D}_1^{\mathrm{I}}$ contain the same columns, yet possibly in a different sequence. In the

transformation from Λ^{I} to Λ^{IV}, only the upper $\gamma - f$ rows in Λ^{I} are therefore allowed to be interchanged among each other, and so are the lower $r - \gamma$ rows. Thus, $\boldsymbol{P}$ subdivides into two permutation matrices:

$$\boldsymbol{P} = \begin{pmatrix} \boldsymbol{P}_1 & \boldsymbol{O} \\ \boldsymbol{O} & \boldsymbol{P}_2 \end{pmatrix}. \tag{4.21}$$

Inserting (4.10), (4.18), and (4.21) into (4.19), we obtain

$$\boldsymbol{I} = \boldsymbol{P}_2 \boldsymbol{Q}. \tag{4.22}$$

Since permutation matrices are orthogonal, (4.22) implies that $\boldsymbol{Q} = \boldsymbol{P}_2^{\mathrm{T}}$. Accordingly, $\boldsymbol{Q}$ is a permutation matrix as well, which completes the justification of step (3).

Step (4) results in a straightforward way from condition (C2).

5. An example

We now analyze the biochemical network depicted in fig. 1 by the developed algorithm. The stoichiometric matrix of this network reads

$$\boldsymbol{C} = \begin{array}{c} \begin{matrix} \boldsymbol{C}_1^* & & & & & \boldsymbol{C}_2^* & & & & \end{matrix} \\ \left(\begin{array}{ccccc|cc|cc} 1 & -1 & 0 & 0 & -1 & 0 & 0 & 0 & 0 \\ 0 & 1 & -1 & 1 & 0 & 0 & 0 & -1 & 0 \\ 0 & 0 & 2 & 0 & 0 & 0 & 0 & 0 & -1 \\ 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & -1 & 0 & 0 & 0 & 1 & 0 \\ \hline -1 & 0 & -1 & 0 & 0 & -1 & -1 & -1 & 2 \\ 1 & 0 & 1 & 0 & 0 & 2 & 1 & 1 & -2 \\ 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 \end{array} \right) \begin{matrix} \} \boldsymbol{C}^* \\ \\ \\ \\ \\ \\ \\ \\ \end{matrix} \\ \begin{matrix} \boldsymbol{C}_1 & & & & & & & \boldsymbol{C}_2 & & \end{matrix} \end{array} \tag{5.1}$$

and has rank 7. Λ can be chosen to be

$$\lambda = \begin{pmatrix} 0 & 0 & 0 & 1 & 0 & 0 & -1 & 1 & 0 \\ \frac{1}{2} & \frac{1}{2} & \frac{1}{2} & 0 & 0 & 0 & 1 & 0 & 1 \end{pmatrix}^{\mathrm{T}}. \tag{5.2}$$

It cannot be rearranged to give a generalized diagonal matrix. Nevertheless, it contains two null vector rows. Accordingly, the corresponding reaction rates v_5 and v_6 are always zero in steady state.

When the concentrations of ATP, ADP, and AMP are considered to be virtually independent of the network response, i.e. when these metabolites are external ones (denoted by P_1, P_2, and P_3, respectively), the lower three rows of $\boldsymbol{C}$ can be cancelled and we obtain the stiochiometric matrix $\boldsymbol{C}^*$. The corresponding matrix λ^* has rank 4 and can be chosen to be

$$\lambda^* = \begin{pmatrix} 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & 0 & 0 \\ 0 & 0 & 0 & 1 & 0 & 0 & 0 & 1 & 0 \\ \frac{1}{2} & \frac{1}{2} & \frac{1}{2} & 0 & 0 & 0 & 0 & 0 & 1 \end{pmatrix}^{\mathrm{T}} . \tag{5.3}$$

Since the fifth row of λ^* contains zeroes only, reaction 5 again constitutes a strictly detailed balanced subnetwork. Cancelling this row, we obtain a matrix Λ^* which can be rearranged to give a generalized diagonal matrix:

$$\Lambda^* = \begin{pmatrix} 1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & \frac{1}{2} & \frac{1}{2} & \frac{1}{2} & 1 \end{pmatrix}^{\mathrm{T}} . \tag{5.4}$$

The corresponding vector $\boldsymbol{V}$ reads

$$\boldsymbol{V} = (v_6, v_7, v_4, v_8, v_1, v_2, v_3, v_9)^{\mathrm{T}}. \tag{5.5}$$

The diagonal blocks in Λ^* correspond to the following five subnetworks (denoted by the reaction sets): $\{R_6\}$, $\{R_7\}$, $\{R_4, R_8\}$, $\{R_1, R_2, R_3, R_9\}$. Reaction R_7 is strictly detailed balanced whenever the corresponding row in Λ^* fulfills eq. (3.6). This equation takes the form $\ln \tilde{q}_7 = 0$ or

$$P_2 / P_1 = q_7 , \tag{5.6}$$

which is consistent with usual equilibrium conditions for single reactions.

Similarly, reaction 6 is detailed balanced whenever

$$P_2^2 / (P_1 P_3) = q_6 . \tag{5.7}$$

Reaction 4 can be detailed balanced only if reaction 8 is, and *vice versa*. The equilibrium condition reads

$$P_2 / P_1 = q_4 q_8 . \tag{5.8}$$

If this condition is not fulfilled, the subnetwork in question is a so-called futile cycle (cf. [10]) since ATP is perpetually degraded with the only effect of heat production.

The remaining subnetwork $\{R_1, R_2, R_3, R_9\}$ is, under physiological conditions, not detailed balanced. Yet it can be, theoretically, if

$$\sqrt{\tilde{q}_1 \tilde{q}_2 \tilde{q}_3}\ \tilde{q}_9 = 1. \tag{5.9}$$

6. Concluding remarks

In the present paper, we have introduced the concept of strict detailed balancing and derived necessary and sufficient conditions for subnetworks of open chemical reaction networks to be strictly detailed balanced. These conditions can be briefly stated as follows. The net fluxes of all reactions which correspond to those rows in λ (the matrix whose columns span the kernel of the stoichiometric matrix) that contain only zeroes are always zero. The set of remaining reactions can be divided into subnetworks according to the decomposition of matrix Λ given by (4.5). Any one of these subnetworks is strictly detailed balanced if and only if condition (4.8) is fulfilled. This condition can be considered as a generalized Wegscheider condition for the respective subnetwork.

An algorithm for detecting the maximum strictly detailed balanced subnetwork, being the union of all strictly detailed balanced subnetworks, has been developed. In practice, this subnetwork often can be determined rather quickly by inspection of the reaction scheme. However, a computer implementation of the algorithm presented is faster and more reliable than the "graphic" method, especially for large reaction networks.

It should be emphasized that our method is applicable only to systems of reversible reactions, i.e. of reactions with finite, non-zero equilibrium constants. As has been stressed in [5], the generalized mass action kinetics defined in (2.2) comprises, in the case of reversible reactions, the "general mass action kinetics" defined by Horn and Jackson [6]. The latter rate law, however, has the advantage that it describes irreversible reactions as well.

In the paper cited above [6], which in the early seventies pioneered a series of papers dealing with properties of steady states of chemically reacting systems (see, e.g. [21,22]), Horn and Jackson also commented on detailed balance, but without deriving conditions for chemical systems to have this property. Rather, their interest centers upon complex balancing, which means that the net formation rate of each complex (i.e. each group of molecules appearing before or after the reaction arrows) is zero. Since detailed balancing implies complex balancing, the latter notion can be considered a natural generalization of the former but, as Horn and Jackson themselves mentioned ([6], ch. 7), the concept of complex balance has the essential drawback of not being

susceptible to macroscopic observation, because the "concentration" of complexes cannot be measured. Nevertheless, this concept was helpful in the derivation of necessary and sufficient conditions for steady states to be unique and globally asymptotically stable [22]. In the present paper, asymptotic stability of the considered steady states was *a priori* presupposed.

The approach presented seems to be of considerable assistance for the modeling of chemical systems in the case of incomplete knowledge of kinetic parameters, for it allows to predict for any given reaction system which of the reactions are always in equilibrium. Therefore, one is able to select some flux control coefficients which are always zero, namely, all C_k^j with R_j being a reaction belonging to a strictly detailed balanced subnetwork. Moreover, we suggest to define, likewise, $C_j^k = 0$ with R_j having the property mentioned above, since it is intuitively clear that such a reaction cannot exert any control either.

It may be supposed that the decomposition of networks as given in (4.5) has some implications for the control structure even if condition (4.8) is not fulfilled, in that reactions corresponding to block $\Lambda^{(j)}$ do not control, under some additional conditions, any reaction of a subnetwork corresponding to another block $\Lambda^{(k)}$. This point is worth investigating in the future.

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Appendix

The following computer program, written in PASCAL, performs the transformation of matrix $\boldsymbol{C}$ into echelon form, the calculation of matrix λ using the echelon matrix $\boldsymbol{C}'$ and the rearrangement of rows and columns of λ so as to obtain a generalized diagonal matrix. In the first part of this rearrangement, all zero rows of λ are transferred to the bottom of λ.

It is tacitly assumed that the rank of the stoichiometric matrix is less than the number of reactions. For the sake of brevity, the declaration of variables, the input and output of data, as well as the test whether or not condition (3.6) is fulfilled are omitted in the program. The meaning of the variables is as follows.

Input parameters:

n, number of reactants; r, number of reactions; c, stoichiometric matrix $\boldsymbol{C}$.

Output parameters:

rank, rank of matrix $\boldsymbol{C}$; l, matrix λ; f, number of zero rows of λ; p, permutation vector which indicates the rearrangement of columns of $\boldsymbol{C}'$ (p[i] is the column index of

column i in the original matrix C); a, vector of which the ith component indicates the index of the bottom row of the submatrix $\Lambda^{(i)}$; b, vector of which the ith component indicates the index of the last column of $\Lambda^{(i)}$.

```
program balance;
procedure re(s, t: integer) (* row exchange *);
begin for m := 1 to max do
  if matrix = 'ca' then begin
    z := ca[s, m]; ca[s, m] := ca[t, m]; ca[t, m] := z;
  end  else begin
    z := l[s, m]; l[s, m] := l[t, m]; l[t, m] := z;  end;
end;
procedure ce(s, t: integer) (* column exchange *);
begin for m := 1 to max do
  if matrix = 'ca' then begin
    z := ca[m, s]; ca[m, s] := ca[m, t]; ca[m, t] := z;
  end  else begin
    z := l[m, s]; l[m, s] := l[m, t]; l[m, t] := z;  end;
end;
begin
(* Transformation of matrix C into echelon form *)
  for j := 1 to r do p[j] := j;
  matrix := 'ca'; i := 1; k := n;
  while i <= k do begin
    if ca[i, i] = 0 then begin j := i + 1;
        while (ca[i, j] = 0) and (j <= r) do j := j + 1;
        if j = r + 1 then begin
            if i < k then begin max := r; re(i, k);
            end;  k := k - 1;
        end else begin
            max := n; ce(i, j); p[0] := p[j]; p[j] := p[i];
            p[i] := p[0];  end;
    end else begin for m := i + 1 to k do
        for j := i to r do begin
            if ca[m, i] < > 0 then
                cb[m, j] := ca[m, j]*ca[i, i]/ca[m, i] - ca[i, j]
            else cb[m, j] := ca[m, j];  end;
        for m := i + 1 to k do
            for j := i to r do ca[m, j] := cb[m, j];
        i := i + 1;  end;
  end;  rank := k;
```

```
(* Calculation of lambda *)
  for m := rank + 1 to r do
    for h := 1 to r-rank do begin
      if m = h + rank then l[m, h] := 1
      else l[m, h] := 0;  end;
  for j := 1 to r-rank do
    for i := rank downto 1 do begin z := 0;
      for m := i + 1 to rank do
        z := z + ca[i, m]*l[m, j];
      l[i, j] := (-ca[i, rank + j] - z)/ca[i, i];  end;
(* Rearrangement of lambda *)
  matrix := 'l'; i := 1; k := r;
  while i < k do begin h := 1;
    while (h <= r-rank) and (l[i, h] = 0) do h := h + 1;
    if h = r-rank + 1 then begin
      if i < k then begin max := r-rank; re(i, k);
        p[0] := p[i]; p[i] := p[k]; p[k] := p[0];
      end;  k := k - 1;
    end else i := i + 1;  end;
  f := r - k; q := 0;
  for m := 0 to r do begin a[m] := 0; b[m] := 0;  end;
  while a[q] < r - f do begin
    i := a[q] + 1; j := b[q] + 1; exchange := true;
    repeat
      exchange := false; h := r-rank;
      while j <= h do begin m := a[q] + 1;
        while (m <= i) and (l[m, j] = 0) do m := m + 1;
        if m = i + 1 then begin m := a[q] + 1;
          while (m <= i) and (l[m, h] = 0) do m := m + 1;
          if m < i + 1 then begin
            if j < h then begin
              max := r - f; ce(j, h); exchange := true;  end;
          end; h := h - 1;
        end else j := j + 1;  end;
      j := j - 1; k := r - f;
      while i <= k do begin m := b[q] + 1;
        while (m <= j) and (l[i, m] = 0) do m := m + 1;
        if m = j + 1 then begin m := b[q] + 1;
          while (m <= j) and (l[k, m] = 0) do m := m + 1;
          if m < j + 1 then begin
            if i < k then begin
              max := r-rank; re(i, k); exchange := true;
              p[0] := p[i]; p[i] := p[k]; p[k] := p[0];  end;
```

```
                end; k := k - 1;
            end else i := i + 1;
        end; i := i - 1;
    until not exchange;
    q := q + 1; a[q] := i; b[q] := j;  end;
end.
```

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