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A GENERALIZATION OF WEGSCHEIDER'S CONDITION. IMPLICATIONS FOR PROPERTIES OF STEADY STATES AND FOR QUASI-STEADY-STATE APPROXIMATION

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Abstract

A generalization of Wegscheider's condition concerning equilibrium constants in chemically reacting systems is formulated, which is then proved to be a necessary and sufficient condition for detailed balancing. In order to include a large multitude of rate laws, a generalized mass action kinetics is considered which comprises usual mass action kinetics and all reversible enzyme kinetics and which is consistent with basic postulates of irreversible thermodynamics for ideal mixtures. Reaction systems of arbitrary stoichiometry are considered. They may contain reactants with fixed concentrations, as is characteristic for models of biochemical reaction networks. Existence, uniqueness, and global asymptotic stability of equilibrium states for reaction systems endowed with generalized mass action kinetics are proved. Using these results, the generalized Wegscheider condition is shown to be a sufficient criterion for the applicability of the quasi-steady-state approximation.

1. Introduction

A network of (bio)chemical reactions is called detailed balanced if in every steady state all reaction rates vanish and if at least one steady state is accessible for this network [1]. Closed reaction systems are always detailed balanced due to the principle of microreversibility [2]. In biochemistry and chemical engineering, however, usually open reaction systems are studied. Such systems often show a time behavior which, after some transients, settles down to be stationary. These steady states need not coincide with thermodynamic equilibrium.

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In section 3 of this paper, a necessary and sufficient condition for the identity of steady and equilibrium states is derived (cf. eq. (3.22)) which turns out to be a generalization of the well-known Wegscheider condition which is usually given in the form

$$\prod_i (k_i/k_{-i}) = 1, \tag{1.1}$$

where k_i and k_{-i} are the rate constants of forward and reverse reactions, respectively, around a cycle of monomolecular reactions with linear rate laws [3; 4, p. 26]. For the history of formulation and resolution of Wegscheider's paradox, the reader is referred to Hearon [3].

In the present paper, reaction systems of arbitrary stoichiometry are considered which can even encompass "external species" [1,5], i.e. reactants with fixed concentrations. In many kinetic models of reaction networks, especially in models of biochemical systems, the concentrations of some reactants (e.g. substrates, products, or co-factors) are deemed to be time-invariant because of a large supply or regulation of infusion or effusion. Since these reactants are not taken into account in stoichiometric matrices, systems with external species are sometimes called "nonstoichiometric" networks [6], in the sense that they apparently violate conservation of matter.

The restrictive assumption of linear rate laws is relaxed in such a way that any reversible reaction kinetics is admitted which can be stated in terms of the generalized mass action kinetics, defined for ideal mixtures in section 2. The restriction to reversible reactions, that is, to reactions with finite equilibrium constants, means no loss of generality inasmuch as one can assume all reactions to be reversible from a thermodynamic point of view. On the other hand, the irreversibility assumption is, for some reactions, often used in kinetic models since it simplifies the governing differential equation systems.

False equilibria, i.e. states in which all rates vanish but at least one affinity does not, are excluded from investigation by restricting it to non-zero concentrations. Since, generally, true chemical equilibria are globally asymptotically stable, this stability property was even used as an axiom in the theory of chemical reaction systems [2]. There exist, however, some exceptions, e.g. certain reactions in regular solutions [7]. Therefore, we show using Lyapunov's second method in section 4 that equilibrium states of reaction systems with generalized mass action kinetics are globally asymptotically stable within the reaction simplex determined by conservation quantities.

Throughout, we in particular refer to biochemical systems, but the results apply to any chemically reacting mixture provided that it can be described by the formalism used in this paper. Since biochemical reaction networks are characterized by a permanent exchange of matter with their surroundings, equilibrium states seem not

to be relevant to them. However, many biochemical systems show the phenomenon of time hierarchy, i.e. the separation of time constants [4,8]. Although subsisting in non-equilibrium, such systems can contain subnetworks of very fast reactions which, after a negligibly short time period, attain a quasi-equilibrium in the sense that their affinities nearly vanish. This will be formulated more exactly in section 5. The variables of fast subsystems are usually eliminated by the quasi-steady-state approximation [4,8–12] which is justified, however, only if the considered steady state of the fast subsystem is asymptotically stable. This requirement is always met if steady state and equilibrium coincide so that the generalized Wegscheider condition can serve as a sufficient criterion for the quasi-steady-state approximation to be applicable.

2. Systems of reactions obeying a generalized mass action kinetics

Throughout this paper we use, in addition to usual notations of matrix algebra, the symbols explained in what follows. I_i, 0_i, and $0_{i,j}$ $(i, j = 1, 2, \ldots)$ denote the $i \times i$ identity matrix, the i-dimensional null vector, and the $i \times j$ null matrix, respectively. If all the components of an i-vector X are positive (non-negative), we write $X > 0_i$ $(X \geqslant 0_i)$. $\mathbb{R}^i_+$ denotes the positive orthant $\{X \in \mathbb{R}^i : X > 0_i\}$. (diag X) stands for the diagonal matrix containing the components of vector X as diagonal elements. Scalar functions of vectors are defined to be the vectors of the functions of components, e.g. $\ln X = (\ln X_1, \ldots, \ln X_n)^T$, where the superscript T denotes transposition of vectors or matrices.

Consider a system of r chemical reactions with net reaction rates v_j forming the vector

$$V = (v_1, v_2, \ldots, v_r)^T. \tag{2.1}$$

Let P_i denote the external species and m be their number. For substances with varying concentrations, we will use the term "internal species" and the symbol X_i $(i = 1, \ldots, n)$. For simplicity's sake, let X_i (P_i) denote the concentrations of internal (external) species as well as the substances themselves. The concentrations X_i are gathered into an n-vector X. Let C be the stoichiometric matrix of the reaction system considered, and c_{ij} its elements. Under the assumption that the concentrations of metabolites are spatially homogeneous, the time behavior $X(t)$ can be described by the ordinary differential equation system

$$\dot{X} = CV(X) \tag{2.2}$$

(cf. [5,7]). We now specify the functions $v_j(X)$ in such a way that their property to represent rate laws of (bio)chemical reactions is taken into account. Throughout, we use the general expression

$$v_j(X) = G_j(X)\Gamma_j\left[-\ln\left(\prod_{i=1}^{n} X_i^{c_{ij}}/\tilde{q}_j\right)\right], \tag{2.3}$$

where

$$\tilde{q}_j = q_j \Big/ \prod_{i=1}^{m} P_i^{e_{ij}}, \tag{2.4}$$

with q_j being the equilibrium constant of reaction j and e_{ij} denoting the elements of the stoichiometric matrix of the external metabolites. Since all P_i are constant, $\tilde{q}_j$ can be interpreted as an apparent equilibrium constant with respect to the concentrations X_i. The expression in square brackets in (2.3) equals, apart from a constant factor, the affinity A_j of reaction j in ideal gases or solutions [13, p. 34]. In order to ensure the existence of A_j, it is necessary to exclude zero concentrations. The loss of generality due to this restriction will be elucidated in section 4.

The kinetics (2.3) can be derived from postulates of (nonlinear) irreversible thermodynamics if coupling effects (i.e. dependences of fluxes w_j on forces A_k with $k \neq j$) are neglected (cf. [13, ch. 3.1]). In accordance with these postulates, the functions $\Gamma_j(A_j)$ are assumed to have the properties

$$\Gamma_j(A_j) = 0 \qquad \text{if} \qquad A_j = 0 \tag{2.5}$$

and

$$\Gamma_j(A_j) > \Gamma_j(A_j') \quad \text{if and only if} \quad A_j > A_j'. \tag{2.6}$$

The function $G_j(X)$ can express nonlinearities caused, for example, by catalytic effects. It can be assumed to be positive for any $X > 0_n$.

Rate laws derived from kinetic considerations can be formulated in a general form to use

$$v_j(X) = F_j(X)\left[q_j \prod_{i=1}^{n} X_i^{c_{ij}^-} \prod_{i=1}^{m} P_i^{e_{ij}^-} - \prod_{i=1}^{n} X_i^{c_{ij}^+} \prod_{i=1}^{m} P_i^{e_{ij}^+}\right], \tag{2.7}$$

where

$$c_{ij}^- = \begin{cases} -c_{ij} & \text{if } c_{ij} < 0 \\ 0 & \text{if } c_{ij} \geqslant 0 \end{cases} \tag{2.8}$$

and

$$c_{ij}^+ = \begin{cases} c_{ij} & \text{if } c_{ij} > 0 \\ 0 & \text{if } c_{ij} \leqslant 0. \end{cases} \tag{2.9}$$

e_{ij}^- and e_{ij}^+ are calculated from e_{ij} analogously to (2.8) and (2.9), and $F_j(X)$ is a positive function. With the help of the definitions (2.4) and

$$\tilde{F}_j(X) = F_j(X) \prod_{i=1}^{m} P_i^{e_{ij}^+}, \tag{2.10}$$

(2.7) can be written as

$$v_j(X) = \tilde{F}_j(X) \left[\tilde{q}_j \prod_{i=1}^{n} X_i^{c_{ij}^-} - \prod_{i=1}^{n} X_i^{c_{ij}^+} \right]. \tag{2.11}$$

All rate laws of enzymatic reactions (steady state as well as equilibrium models) can be described by (2.11) [11]. In the case of irreversible reactions, the orientations of reactions and, thus, the signs of the elements c_{ij} of C have to be chosen in such a way that $\tilde{q}_j = 0$ rather than $\tilde{q}_j \to \infty$ in order to ensure the existence of the r.h.s. of (2.11).

If only reversible reactions and non-vanishing concentrations are considered, the kinetics arising from (2.11) is a special case of (2.3), as can immediately be seen by choosing

$$\Gamma_j(A_j) = A_j \tag{2.12}$$

and

$$G_j(X) = \tilde{F}_j(X)\tilde{G}_j(X), \tag{2.13}$$

where

$$\tilde{G}_j(X) = \begin{cases} \dfrac{\prod_{i=1}^{n} X_i^{c_{ij}^+} - \tilde{q}_j \prod_{i=1}^{n} X_i^{c_{ij}^-}}{\ln\left(\prod_{i=1}^{n} X_i^{c_{ij}} / \tilde{q}_j \right)} & \text{if } \prod_{i=1}^{n} X_i^{c_{ij}} \neq \tilde{q}_j \qquad (2.14a) \\ \prod_{i=1}^{n} X_i^{c_{ij}^+} & \text{if } \prod_{i=1}^{n} X_i^{c_{ij}} = \tilde{q}_j . \qquad (2.14b) \end{cases}$$

The r.h.s. of (2.14a) has a discontinuity across the manifold

$$\prod_{i=1}^{n} X_i^{c_{ij}} = \tilde{q}_j$$

which is removed by the definition (2.14b). As $G_j(X)$ is assumed to have no zeroes, we have, by virtue of (2.5) and (2.6),

$$v_j(X) = 0 \quad \text{if and only if} \quad \prod_{i=1}^{n} X_i^{c_{ij}} = \tilde{q}_j \,, \tag{2.15}$$

which is the well-known law of mass action. The usual mass action kinetics

$$v_j(X) = \vec{k}_j \prod_{i=1}^{n} X_i^{c_{ij}^-} - \overleftarrow{k}_j \prod_{i=1}^{n} X_i^{c_{ij}^+} \,, \tag{2.16}$$

where $\vec{k}_j$ and $\overleftarrow{k}_j$ are the rate constants of forward and reverse reactions, respectively, is not, of course, the only kinetics obeying this law. Since (2.16) is a special case of kinetics (2.3) provided that neither $\vec{k}_j$ nor $\overleftarrow{k}_j$ vanishes, we term (2.3) "generalized mass action kinetics". Under the mentioned condition concerning $\vec{k}_j$ and $\overleftarrow{k}_j$, it also comprises the "general mass action kinetics" considered by Horn and Jackson [1] which admits matrices C disobeying the law of mass conservation or having non-integer elements, and which does not restrict $\vec{k}_j$ and $\overleftarrow{k}_j$ to satisfy the principle of microreversibility. That kinetics, however, cannot allow for allosteric, competitive, cooperative, and other nonlinear effects, which are often observed in biochemical kinetics and which can be described by the functions $\tilde{F}_j(X)$ in (2.11) or $G_j(X)$ in (2.3).

3. A generalization of Wegscheider's condition

3.1. GENERAL THEORY

In steady states, eq. (2.2) takes the form

$$CV(X^\star) = 0_n \,, \tag{3.1}$$

where the asterisk denotes steady-state conditions. Of course, eq. (3.1) is fulfilled if

$$V(X^\star) = 0_r \,. \tag{3.2}$$

We now investigate under which conditions (3.2) follows from (3.1). Let

$$c = \operatorname{rank}(C). \tag{3.3}$$

We rearrange the rows and columns of C in such a way that this matrix can be partitioned into four submatrices:

$$C = \begin{pmatrix} C_1 & C_2 \\ C_3 & C_4 \end{pmatrix} \,, \tag{3.4}$$

where C_1 is a nonsingular $c \times c$ matrix. Since the rows of $(C_3 \;\; C_4)$ are then linearly dependent on the rows of $(C_1 \;\; C_2)$, eq. (3.1) is equivalent to

$$(C_1 \;\; C_2)\, V(X) = 0_c \,. \tag{3.5}$$

Now we can distinguish the two cases $c = r$ and $c < r$:

(i) $c = r$. Equation (3.5) reads $C_1 V = 0_r$, which immediately implies eq. (3.2) due to the regularity of C_1.

(ii) $c < r$. In this case, there exist $r - c$ linear dependences between the columns of C, which can be written as follows

$$C\lambda = 0_{n,r-c} \,, \tag{3.6}$$

with λ being an $r \times (r - c)$ matrix with rank $r - c$. In accordance with the decomposition (3.4) of C, λ can be chosen to be

$$\lambda = \begin{pmatrix} \lambda_0 \\ I_{r-c} \end{pmatrix}, \tag{3.7}$$

where

$$\lambda_0 = -C_1^{-1} C_2 \,. \tag{3.8}$$

C_1^{-1} exists because of the regularity of C_1.

We now define the following vectors

$$G(X) = (G_1(X), \ldots, G_r(X))^{\mathrm{T}} \tag{3.9}$$

$$A = (A_1, \ldots, A_r)^{\mathrm{T}} \tag{3.10}$$

$$\Gamma(A) = (\Gamma_1(A_1), \ldots, \Gamma_r(A_r))^{\mathrm{T}} \tag{3.11}$$

so that we can write eq. (2.3) in the form

$$V(X) = [\mathrm{diag}\, G(X)]\; \Gamma(\ln \tilde{q} - C^{\mathrm{T}} \ln X). \tag{3.12}$$

Since all $\Gamma_j(A_j)$ are monotonic increasing functions, there exist inverse functions $\Gamma_j^{-1}(\cdot)$ which we can combine with the vector $\Gamma^{-1}(\cdot)$. Thus, we can transform eq. (3.12) into

$$\ln \tilde{q} - C^{\mathrm{T}} \ln X = \Gamma^{-1} \{[\mathrm{diag}\, G(X)]^{-1} V(X)\}. \tag{3.13}$$

Multiplying this equation by V^T from the left, one obtains in the steady state

$$V^T \ln \tilde{q} = V^T \Gamma^{-1} \left\{ \left[\text{diag} \ \frac{1}{G(X)} \right] V(X) \right\} \tag{3.14}$$

$$= \sum_{j=1}^{r} \upsilon_j \Gamma_j^{-1}(\upsilon_j / G_j). \tag{3.15}$$

As $\Gamma_j(A_j)$ is a monotonic increasing function passing through the origin of coordinates, so is $\Gamma_j^{-1}(\cdot)$, which implies that its function values have the same sign as its arguments. Furthermore, since $G_j(X) > 0$, we may write

$$\Gamma_j^{-1}(\upsilon_j / G_j) = \rho_j \upsilon_j , \tag{3.16}$$

where ρ_j is a function of X with the property

$$\rho_j(X) > 0 \quad \text{for any } X. \tag{3.17}$$

Now we can write eq. (3.15) as follows

$$V^T \ln \tilde{q} = \sum_{j=1}^{r} \rho_j \upsilon_j^2 . \tag{3.18}$$

Equations (3.17) and (3.18) give

$$V^T \ln \tilde{q} \geqslant 0 \tag{3.19}$$

with equality if and only if $V = 0_r$. In this case (i.e. at thermodynamic equilibrium), eq. (3.13) leads to

$$\ln \tilde{q} - C^T \ln X = 0_r . \tag{3.20}$$

Multiplication of eq. (3.20) by λ^T from the left yields, under consideration of (3.6),

$$\lambda^T \ln \tilde{q} = 0_{r-c} , \tag{3.21}$$

which can be written as

$$\prod_{j=1}^{r} \tilde{q}_j^{\lambda_{jk}} \equiv \prod_{j=1}^{r} \left[q_j \Big/ \prod_{i=1}^{m} P_i^{e_{ij}} \right]^{\lambda_{jk}} = 1, \quad k = 1, \ldots, r-c. \tag{3.22}$$

Equation (3.21) is a necessary condition for $V = 0_r$ to be possible. We now prove that (3.21) is even sufficient for the equivalence of steady state and equilibrium. Since λ fulfills, by definition, eq. (3.6) and all its columns are linearly independent, these columns taken as vectors span the subspace kernel (C) being the space of all vectors V fulfilling eq. (3.1). These can, therefore, be represented in the form

$$V = \lambda Z \tag{3.23}$$

with $(r - c)$-dimensional vectors Z. By multiplying eq. (3.13) by V^T from the left and inserting (3.23), we obtain, under steady-state conditions,

$$V^T \Gamma^{-1} \left\{ \left[\operatorname{diag} \frac{1}{G(X)} \right] V(X) \right\} = Z^T \lambda^T \ln \tilde{q} . \tag{3.24}$$

If condition (3.21) is fulfilled, the r.h.s. of (3.24) vanishes so that we may write, using eq. (3.16),

$$\sum_{j=1}^{r} \rho_j v_j^2 = 0. \tag{3.25}$$

This equation implies, due to inequality (3.17), $v_j = 0$ for all j, which completes the proof. We formulate the obtained result in the following theorem.

THEOREM 1

For any reaction network endowed with generalized mass action kinetic form (2.3), each steady state with no concentration vanishing is identical with a thermodynamic equilibrium state, i.e. $CV(X^\star) = 0_n \Leftrightarrow V(X^\star) = 0_r$ $(X^\star > 0_n)$, if and only if either the rank of stoichiometric matrix C equals the number of reactions, r, or condition (3.21) is fulfilled.

3.2. EXAMPLES AND EXPLANATORY REMARKS

The statement of theorem 1 becomes clearer with the help of the following examples. First, we consider a cycle of n monomolecular reactions with the stoichiometric matrix

$$C = \begin{pmatrix} -1 & 0 & 0 \ldots 0 & 1 \\ 1 & -1 & 0 \ldots 0 & 0 \\ 0 & 1 & -1 \ldots 0 & 0 \\ 0 & 0 & 1 \ldots 0 & 0 \\ \cdot & \cdot & \cdot \quad \cdot & \cdot \\ \cdot & \cdot & \cdot \quad \cdot & \cdot \\ \cdot & \cdot & \cdot \quad \cdot & \cdot \\ 0 & 0 & 0 \ldots 1 & -1 \end{pmatrix} \tag{3.26a}$$

or, written in a more abstract way using Kronecker δ,

$$c_{ij} = -\delta_{ij} + \delta_{i,j+1} + \delta_{i,1}\,\delta_{j,n}, \quad i,j = 1,\ldots,n. \tag{3.26b}$$

Since rank$(C) = n - 1$, λ contains one column which can be chosen to be

$$\lambda = (1 \;\; 1 \;\ldots\; 1)^{\mathrm{T}} \tag{3.27}$$

so that eq. (3.22) takes the form

$$\prod_{j=1}^{r} q_j = 1. \tag{3.28}$$

Equation (3.28) is the well-known Wegscheider condition [3; 4, p. 26]. It was originally formulated as a relation among rate constants (cf. eq. (1.1)) in order to conform the mass action expression as obtained from chemical kinetics to thermodynamics. For a reaction system without external species and having the stoichiometric matrix

$$C = \begin{pmatrix} -1 & 0 & 2 \\ 1 & -1 & 0 \\ 0 & 1 & -2 \end{pmatrix}, \tag{3.29}$$

for example, condition (3.22) takes the form

$$q_1^2\, q_2^2\, q_3 = 1, \tag{3.30}$$

which is not a special case of Wegscheider's condition (3.28). Moreover, (3.22) is more general than (3.28) inasmuch as the existence of external species is admitted, which is reflected in the replacement of "real" equilibrium constants q_j by apparent constants $\tilde{q}_j$.

In closed systems, relation (3.21) is always fulfilled since any chemical reaction system without external species tends to thermodynamic equilibrium. It can then be considered as a test criterion for the accuracy of measured rate or equilibrium constants. Relations between equilibrium constants have been given earlier [14, p. 171; 15, p. 259], but we are not aware of such a compact and general formulation as that in (3.21).

In equilibrium thermodynamics, the mutual dependences of equilibrium constants need not be considered at all if, as is often done, only c = rank(C) reactions (called independent reactions) are chosen for investigation in such a way that their corresponding columns in C are linearly independent. In non-equilibrium, the dependent reactions cannot be neglected because they affect the temporal system behaviour.

Typical examples of systems with dependent reactions are networks containing cycles characterized by (3.26). In biochemistry, reaction cycles are of great importance (citric acid cycle, glyoxalate cycle, urea cycle, etc. (cf. [16])), but they are not composed of monomolecular reactions only. Consider, for instance, the cycle formed by the enzymes hexokinase and glucose-6-phosphatase (cf. [16]). If $X_1, X_2, \ldots, X_6$ denote the substances glucose, glucose-6-phosphate, adenosine triphosphate (ATP), adenosine diphosphate (ADP), inorganic phosphate (P_i), and water, respectively, the topology of this reaction system is given by the matrix

$$C = \begin{pmatrix} -1 & 1 \\ 1 & -1 \\ -1 & 0 \\ 1 & 0 \\ 0 & 1 \\ 0 & -1 \end{pmatrix} \tag{3.31}$$

which has full rank. However, if ATP, ADP, P_i, and water are considered as external metaboliyes (as is often done), C reads

$$C = \begin{pmatrix} -1 & 1 \\ 1 & -1 \end{pmatrix} \tag{3.32}$$

and $c < r$ certainly holds. Systems of reactions representing one and the same transformation of metabolites catalyzed by several enzymes can be regarded as cycles as well. They are characterized by $c < r$, anyway. Furthermore, the fixation of concentrations, i.e. the conversion of internal species into external ones, can cause c to be smaller than r even in acyclic systems. This can be seen by means of the reaction sequences

$$\text{(a)} \quad P_1 \rightarrow X_1 \rightarrow P_2; \qquad \text{(b)} \quad P_1 \rightarrow X_1 \begin{matrix} \nearrow P_2 \\ \searrow P_3 \end{matrix},$$

where $c = 1$, $r = 2$ (a), and $c = 1$, $r = 3$ (b). If λ is chosen according to eq. (3.7), we have

$$\text{(a)} \quad \lambda = \begin{pmatrix} 1 \\ 1 \end{pmatrix} \qquad \text{(b)} \quad \lambda = \begin{pmatrix} 1 & 1 \\ 1 & 0 \\ 0 & 1 \end{pmatrix}. \tag{3.33a,b}$$

These examples illustrate the above-mentioned fact that the columns of λ span the space of all vectors V which fulfill eq. (3.1).

In open systems, eq. (3.21) implies restrictions concerning the concentrations P_i which are not always fulfilled. It can then be used as a necessary and sufficient condition for detailed balancing.

4. Existence, uniqueness, and stability of equilibrium states

If $c = r$, the row vectors of C span the whole space $\mathbb{R}^r$ so that $\ln \tilde{q}$ can be represented as a linear combination of rows of C:

$$\ln \tilde{q} = C^{\mathrm{T}} Y, \tag{4.1}$$

with $Y \in \mathbb{R}^n$. If $c < r$ and eq. (3.21) hold, $\ln \tilde{q}$ must be contained in the kernel of λ^{T} having dimension c and being spanned by row vectors of C due to eq. (3.6) and $\operatorname{rank}(C) = c$. Hence, eq. (4.1) holds also in the case $c < r$. We choose a concentration vector $\bar{X}$ to be $\bar{X} = \exp(Y)$. It lies in $\mathbb{R}_+^n$ and fulfills, by virtue of eq. (4.1), the equation

$$C^{\mathrm{T}} \ln \bar{X} = \ln \tilde{q}, \tag{4.2}$$

which is equivalent to eq. (3.20) and, hence, to $V(\bar{X}) = 0_r$. Thus, $\bar{X}$ is a vector of equilibrium concentrations.

In the case $c = n$, this equilibrium state is even unique since the n upper equations of vector equation (4.2) determine $\bar{X}$ uniquely. In the case $c < n$, there exist $n - c$ independent conservation conditions restricting reactant concentrations, which can be written in the form

$$\gamma X(t) = K, \tag{4.3}$$

where γ is an $(n - c) \times n$ matrix fulfilling

$$\gamma C = 0_{n-c,\,r} \tag{4.4}$$

and having rank $n - c$ (cf. [5]). K denotes an $(n - c)$-dimensional vector of constants.

Due to eq. (4.3) and the non-negativity of concentrations, the trajectory $X(t)$ is restricted to a subset of $\mathbb{R}^n$

$$\Sigma = \{X \in \mathbb{R}^n : X \geqslant 0_n, \gamma X = K\}, \tag{4.5}$$

which is called reaction simplex [1] or concentration polyhedron [5]. We can assume Σ to have dimension c since, otherwise, some X_i would vanish for all t. We elucidate this statement by means of an exemplifying reaction having stoichiometric matrix

$$C = \begin{pmatrix} -1 \\ 1 \end{pmatrix} \tag{4.6}$$

and endowed with the kinetics associated with

$$v_1 = k_1 X_1 X_2 - k_{-1} X_2^2 , \tag{4.7}$$

which is also considered by Othmer [7]. γ can be chosen to be $\gamma = (1\ \ 1)$ so that the reaction simplex is given by $X_1, X_2 \geqslant 0$, $X_1 + X_2 = \text{const}$. This simplex has dimension one unless the conservation sum vanishes, in which case both the concentrations vanish as well. As stated in section 2, we exclude all cases with zero concentrations. This means no loss of generality if some X_i identically vanish (i.e. $X_i(t) \equiv 0$ for all t) due to special values of conservation quantities. In this case, the reactants in question can be deleted and one can work in a lower-dimensional phase space. However, this case is not the only one characterized by $X_i(t) \equiv 0$ for some i. In the above example, all states with $X_1 > 0$, $X_2 = 0$ are stationary and even equilibrium states if equilibrium is defined in the sense of vanishing rates. Yet, since affinity is not equal to zero, they represent "false equilibria" [7] and are not stable. By excluding vanishing concentrations, we circumvent the problem of false equilibria which is, however, worth being investigated in more detail in the future.

Finally, one has to consider the case that some X_i vanish non-identically, i.e. only for some t. It can easily be shown that this is possible solely for the initial point of time, say $t = 0$. Since we are interested in the asymptotic time behavior, we can restrict the analysis to $t > 0$, so that the assumption about non-vanishing concentrations does not imply a loss of generality in this case.

Returning to the equilibrium vector $\bar{X} = \exp(Y)$, we can evaluate the vector K belonging to $\bar{X}$ using eq. (4.3). Thus, at least for one vector K, an equilibrium state does exist. In what follows, we show, with the help of Lyapunov's second method [17], being a standard technique in chemical kinetics [1,2,5,7,13], that for any $(n - c)$-dimensional vector K allowing positive concentrations, a unique equilibrium vector exists. Following Horn and Jackson [1], we first define a function $H(X)$ in $\mathbb{R}_+^n$ by

$$H(X) = \sum_{i=1}^{n} X_i(\ln X_i - \ln \bar{X}_i - 1), \tag{4.8}$$

where $\bar{X}_i$ are the components of $\bar{X}$. Obviously, $H(X)$ is continuously differentiable at least once everywhere in $\mathbb{R}_+^n$. Σ and $H(X)$ are easily seen to be a convex set and a strictly convex function, respectively. Therefore, and due to the behavior of $H(X)$ at infinity and in the limit $X_i \to 0$ for some i, $H(X)$ can be shown to assume its minimum relative to Σ at a unique point in the interior of Σ [1], which we denote by $\hat{X}(K)$. Due to the differentiability of $H(X)$, the necessary condition for the existence of a minimum

$$\text{grad}\, H(X) + \gamma^{\mathrm{T}} \nu = 0_n \tag{4.9}$$

must be fulfilled at $\hat{X}$, where ν denotes the vector of Lagrangian multipliers by which the conservation constraint (4.3) is taken into account.

From eq. (4.8) we obtain

$$\operatorname{grad} H(X) = \ln X - \ln \bar{X} \,. \tag{4.10}$$

Multiplying this equation by C^{T} from the left for $X = \hat{X}$, we get, by virtue of eqs. (4.2), (4.4), and (4.9),

$$C^{\mathrm{T}} \ln \hat{X} = \ln \tilde{q} \,, \tag{4.11}$$

from which eq. (3.2) with $X^{\star} = \hat{X}$ follows. Thus, the vector $\hat{X}(K)$ is an equilibrium point for given K.

Now we define the strictly convex function

$$L(X) = H(X) - H(\hat{X}), \tag{4.12}$$

which has the property $L(\hat{X}) = 0$. It is related to the Gibbs free energy [2]. The time derivative of $L(X)$ along a solution $X(t)$ of (2.2) reads

$$\dot{L} = \dot{X}^{\mathrm{T}} \operatorname{grad} H(X). \tag{4.13}$$

With the help of eqs. (2.2) and (4.9), we get

$$\dot{L} = V^{\mathrm{T}} C^{\mathrm{T}} (\ln X - \ln \bar{X}), \tag{4.14}$$

which can be written as

$$\dot{L} = -V^{\mathrm{T}} \Gamma^{-1} \left\{ \left[\operatorname{diag} \frac{1}{G(X)} \right] V(X) \right\} \tag{4.15}$$

using eqs. (3.13) and (4.2). Due to (3.16) and (3.17), we have

$$\dot{L} \leqslant 0 \tag{4.16}$$

with equality if and only if $X = \hat{X}(K)$. Thus, $L(X)$ possesses all properties of a Lyapunov function of the differential equation system (2.2) in the interior of Σ and with respect to the singular point $\hat{X}$ [17]. Therefore, the equilibrium point $\hat{X}(K)$ is globally asymptotically stable relative to the interior of the considered reaction simplex Σ (i.e. for the given vector K or given initial conditions $X(0)$), and is, therefore, even the unique (true) equilibrium point for given K. The main result of this section is stated in the following theorem.

THEOREM 2

If the rate laws of all reactions of a reaction system can be described by (2.3), and either the rank of its stoichiometric matrix equals the number of reactions or condition (3.21) is fulfilled, there exists, for each vector K of conservation quantities, a unique equilibrium state with finite concentrations. This state is globally asymptotically stable relative to the interior of the reaction simplex corresponding to K.

5. Implications for quasi-steady-state approximation

The quasi-steady-state approximation (QSSA), which is based on Tikhonov's theorem (cf. [18, p. 251]), is frequently employed to simplify the integration of the differential equation system (2.2) if it is stiff [4,8–12], which is often the case, particularly in biochemical reaction networks. Roughly speaking, one can distinguish two variants of QSSA. The classical stationary-state hypothesis, which was first developed by Bodenstein (cf. [9, p. 50]), is applied to systems with widely different reactant concentrations, whereas the second variant, also termed "rapid-equilibrium approximation" [4, p. 43], is based on the normalization of strongly separated reaction rates and necessitates a linear transformation of variables (theoretical background in [8,10,11], applications to biochemical systems in [8,19]). If a separation of concentrations as well as of reaction rates occurs, it is possible to apply both the mentioned variants. In enzyme kinetics, for example, steady-state and equilibrium models have been derived which correspond to Bodenstein's method and rapid-equilibrium approximation, respectively.

For brevity's sake, we use formulas for the latter type of QSSA only. Following Schauer and Heinrich [11], we renumber the reactions and, accordingly, the columns of C such that

$$V = \begin{pmatrix} U \\ W \end{pmatrix} \tag{5.1}$$

and

$$|u_i| \ll |w_j| \quad \text{for all } i, j \tag{5.2}$$

hold, where u_i and w_j are the components of U and W, respectively. Since U and W depend on X, one has to indicate in which region of $\mathbb{R}^n_+$ inequality (5.2) is to hold. Since Tikhonov's theorem is formulated for bounded domains $D \subset \mathbb{R}^n$, it is favorable to restrict the validity of (5.2) to such a domain, except for a small neighborhood of the submanifold defined by $W = 0_p$ (the "slow submanifold"), where p denotes the dimension of W.

Let R be the matrix composed by those columns of C which correspond to U, and S the matrix of the remaining columns of C:

$$C = (R \quad S). \tag{5.3}$$

The submatrix S represents, after deletion of all rows containing zeroes only, the stoichiometric matrix of the "fast subsystem" [11], also termed "steady-state subnetwork" [10]. For the following calculations, it is of no importance whether or not these rows in S are canceled so that we identify S with the stoichiometric matrix of the fast subsystem.

We normalize W by

$$\tilde{W} = \mu W, \tag{5.4}$$

where μ is chosen such that

$$\max_i |u_i| \cong \min_j |\tilde{w}_j| \tag{5.5}$$

everywhere in the region of validity of (5.2). Then (2.2) transforms to the singularly perturbed system

$$\dot{X} = RU(X) + \frac{1}{\mu} S\tilde{W}(X). \tag{5.6}$$

From the premises of Tikhonov's theorem, one can conclude that for the steady-state assumption to be justified it is necessary that the differential equation system

$$\frac{dX}{d\tau} = S\tilde{W}(X) \tag{5.7}$$

has at least one asymptotically stable singular point (corresponding to a stable steady state of the fast subsystem), and that the initial concentration values lie in its domain of influence. Asymptotic stability is considered within that reaction simplex of the fast subsystem which is given by the conservation quantities. These are composed by the conservation quantities of the whole reaction system and the so-called "slow moieties" or "pool variables" [8,11] obtained by the above-mentioned variable transformation [10].

For a wide class of systems, QSSA can be shown to be applicable using theorem 2. If we replace, in eq. (5.7), S, $\tilde{W}$, and τ by the symbols C, V, and t, respectively, we see that the above-mentioned preconditions for the application of QSSA are always fulfilled if the fast subsystem satisfies the generalized Wegscheider condition (3.21) or if its stoichiometric matrix has only linearly independent columns. The latter requirement is met in many models of biochemical systems (cf. the exemplifying system in [11] and the model in [19]). In both cases, eq. (5.7) has, for each vector of conservation quantities, exactly one singular point which is globally asymp-

totically stable, so that any initial point $X(0) > 0_n$ lies, of course, in its basin of attraction.

The remaining premises of Tikhonov's theorem (continuity of right-hand sides of differential equations and existence and uniqueness of trajectories) are always fulfilled for equations describing deterministic chemical kinetics.

The statement that the fast subsystem is in equilibrium holds for $\mu \to 0$, $\tau \to \infty$. A comparison of eqs. (5.6) and (5.7) shows that τ can be considered as a stretched time-scale t/μ. In time-scale t, $\tilde{W} = 0_p$ reads

$$\lim_{\mu \to 0} \mu W = 0_p,$$

which need not imply $W = 0_p$. This explains the fact alluded to in the introduction that a subsystem can subsist in equilibrium (which is, in real systems, only a quasi-equilibrium, since μ can be very small but cannot vanish) although a net flux through this system exists. The term "equilibrium reaction" often used in biochemistry has to be interpreted in this way. One has, however, to bear in mind that "fast reaction" and "equilibrium reaction" do not necessarily coincide.

The variant of QSSA which is suited for systems with widely different concentrations can be investigated in the light of theorem 2 as well. Yet the hypotheses of this theorem are seldom fulfilled in this case, so that other mathematical tools, e.g. the zero deficiency theorem [6] (for other stability theorems, see Clarke [5]), have to be employed in order to check whether singular points of fast subsystems are stable. This was done, for example, by Battelli and Lazzari [12] concerning steady-state enzyme kinetics.

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