

A GRAPH-THEORETIC ANALYSIS OF CHEMICAL REACTION NETWORKS

I. Invariants, Network Equivalence and Nonexistence of Various Types of Steady States*

Hans G. Othmer
Department of Mathematics
University of Utah
Salt Lake City, Utah 84112

1981

*Based on Rutgers University Course Notes, 1979

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1 Abstract

The dynamical behavior of a chemically-reacting system is determined by the stoichiometry of the reactions, the structure of the graph underlying the network, and the reaction phenomenology embodied in the rate laws. Herein we develop a new approach to the analysis of networks, using graph-theoretic techniques, that separates the individual influences to the extent possible, and facilitates the analysis of their interaction. We show how the reaction invariants are related to the stoichiometry and the network structure and give sufficient conditions under which the reaction simplex is not compact. The notion of dynamical equivalence of networks is defined and three types of equivalence transformations are introduced. In particular, it is shown that a network of positive deficiency, a term defined later, is dynamically equivalent to one with zero deficiency. In our approach, the steady states of any network fall into three classes, and conditions are given under which each of these classes is empty.

2 Introduction

The objective in a qualitative analysis of a dynamical system described by an evolution equation of the form $\dot{u} = F(u, p)$, where u is the state and p is a parameter vector, is to predict the qualitative evolution of u for different initial conditions, and to determine how the evolution depends on the parameters. The basic problem is the same, irrespective of whether the equation arises from a problem in chemical reaction dynamics, ecological interactions between species, membrane transport or a variety of similar problems. However, the constraints inherent in a particular problem, such as the non-negativity of u or of some of the parameters, may place additional constraints on the structure of the equations,

and such constraints may make the general analysis of wide class of problems feasible. This is particularly true for problems of the sort mentioned, for in these the pattern of 'reaction' between the various species imposes a great deal of structure on the governing equations. Our objective here is to develop a general graph-theoretic approach to the analysis of the equations that describe open reacting systems, an approach that makes systematic use of the structure dictated by the reactions. The general ideas can be applied to a variety of other problems, as examples in sequels to this paper will show.

Two fundamental properties of closed reacting systems are crucial to the analysis of their equilibrium and dynamic behavior. Firstly, the fact that the mass of the mixture is constant implies that the reaction simplex, a term defined later, is compact, and therefore all closed systems have a least one equilibrium point. Secondly, the hypothesis that the dissipation is non-negative and vanishes only when the net rate of all reactions vanish implies that all trajectories that begin in the interior of the simplex approach an equilibrium point as $t \rightarrow \infty$. In an open system, which by definition can exchange material with its environment, reaction invariants such as the total mass need not be time-independent and there is no *a priori* guarantee that a steady state exists. Even if one does exist, the dissipation is generally not minimized at the steady state, as the following argument shows.

Suppose that a system is spatially uniform and that its dynamics are described by the solution of

$$\frac{dc}{dt} = J(c, c^o) + \bar{\nu}R(c),$$

where $J(c, c^o)$ is the exchange flux between the system and a time-invariant bath, $\bar{\nu}$ is the usual stoichiometric matrix (Aris 1965) and $R_i(c)$ is the intrinsic rate of the i th reaction. The free energy per unit volume of the system is $G = \langle \mu, c \rangle$, where μ is the chemical potential vector,¹ and its rate of change along trajectories of the system is given by

$$\begin{aligned} \frac{dG}{dt} &= \langle \mu, J \rangle - \langle \bar{\nu}^T \mu, R \rangle \\ &= \langle \mu^o, J \rangle - \{ \langle \mu^o - \mu, J \rangle + \langle \bar{\nu}^T \mu, R \rangle \} \equiv \langle \mu^o, J \rangle + \Phi \end{aligned}$$

The first term represents the free energy flux into the system and Φ is the internal dissipation due both to reaction and transport. At a steady state the flux balances the dissipation, but there is no *a priori* reason why either G or Φ should have a local minimum at a steady state. As Denbigh (1952) first pointed out, it would be fortuitous if the solution of the system

$$\frac{\partial \Phi}{\partial c_i} = 0 \quad i = 1, \dots, n$$

coincided with the solution of

$$0 = J(c, c^o) + \bar{\nu}R(c).$$

It happens that they do coincide when the rate expressions are linear functions of the affinities, the phenomenological coefficients are constant, and the Onsager relations are satisfied (DeGroot and Mazur 1962). In this case it is easy to show that $d\Phi/dt \leq 0$. However, this so-called 'evolution criterion' generally fails even in a closed system, as an example in Othmer (1981) shows. There are Lyapunov functions for certain classes of open system (see the Discussion section), but in general there is no universal evolution criterion for open system comparable to ' $dG/dt \leq 0$ ' for closed systems, as Mel and Ewald (1974) have shown. Thus the analysis of open systems necessarily proceeds on more of a case-by-case basis, but it is desirable to identify general classes of mechanisms for which the dynamical behavior can readily be determined. The exchange flux $J(c, c^o)$ can formally be viewed as the result of a reaction $\mathcal{M}^0 \rightleftharpoons \mathcal{M}$ that 'converts' a species at one concentration to the same species at a different concentration, and from this viewpoint, the different modes of transport, such as convection in a CSTR or facilitated transport across a membrane, differ primarily

¹Here and hereafter \langle, \rangle denotes the Euclidean inner product on R_n .

in the 'mechanism' of the exchange reaction. We shall adopt this viewpoint here and use the word mechanism in the extended sense.

In the following section we introduce the graph-theoretic formulation of the governing equations and some elementary concepts and facts from graph theory. Section 3 deals with the existence of invariants and the compactness of the reaction simplex. In the fourth section we define the notion of dynamical equivalence of networks and show that every network is dynamically equivalent to one with zero deficiency. The fifth section deals with the existence problem for steady-state solutions. A discussion of related work is given in the concluding section.

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3 How Stoichiometry and Network Structure are Reflected in the Dynamical Equations.

3.1 The Graph Associated with a Reaction Network

Suppose that the reacting mixture contains n chemical species \mathcal{M}_i , which may be atoms, ions or molecules, and let ν_{ij} be the stoichiometric coefficient of the i^{th} species in the j^{th} reaction. The ν_{ij} are non-negative integers that represent the normalized molar proportions of the species in a reaction. Each reaction is written in the form

$$\sum_i^{react.} \nu_{ij} \mathcal{M}_i = \sum_i^{prod} \nu_{ij} \mathcal{M}_i \quad j = 1, \dots, r, \quad (1)$$

where the sums are over reactants and products, respectively in the j^{th} reaction. Our convention differs slightly from the usual one in which reactions are written (Aris 1965).

$$\sum_i \nu_{ij} \mathcal{M}_i = 0$$

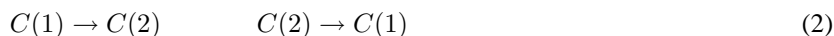
and the stoichiometric coefficients of reactants are negative.

If the reaction at (1) represents an event that actually occurs at the molecular level it is said to be an elementary reaction and otherwise it is called a compound reaction. A mechanism for a compound reaction comprises one or more elementary reactions. Once the mechanism for each of the reactions under consideration is fixed, the significant entities so far as the network topology is concerned are not the species themselves, but rather the linear combinations of species that appear as reactants or products in the various elementary steps. Following Horn and Jackson (1972), these linear combinations of species will be called *complexes*; clearly a species may also be a complex. We usually assume that all reactions in the set under consideration are elementary unless some explicit reductions, such as those to be described shortly, have been made. We further assume that the temperature and pressure of the mixture are held constant and that the volume changes accompanying reaction are negligible. Thus the state of the system is specified by the concentration vector $c = (c_1, \dots, c_n)^T$ and this must lie in R_n^+ , the non-negative 'orthant' of an n -dimensional real vector space.

To allow consideration of irreversible reactions, the forward and reverse reaction of a reversible pair will be considered separately; thus a reaction of the form



will be represented by the pair



where $C(1) \equiv \mathcal{M}_1 + \mathcal{M}_2$ and $C(2) \equiv \mathcal{M}_3$.

Let $\mathcal{M} = \{\mathcal{M}_1, \dots, \mathcal{M}_n\}$ be a set of species, let $\hat{\mathcal{M}}$ be the set of formal linear combinations with integral coefficients of the species, and let $\mathcal{C} = \{C(1), \dots, C(p)\}$ be a set of complexes. A *reaction network* consists of the triple $\{\mathcal{M}, \hat{\mathcal{M}}, \mathcal{C}\}$, together with a stoichiometric function $\hat{\nu} : \hat{\mathcal{M}} \rightarrow \mathcal{C}$ and a binary relation $\mathbb{R} \subset \mathcal{C} \times \mathcal{C}$. The function $\hat{\nu}$, which identifies a linear combination of species as a complex is onto, and the relation \mathbb{R} has the following properties:

- (i) $(C(i), C(j)) \in \mathbb{R}$ if and only if there exists one and only one reaction of the form $C(i) \rightarrow C(j)$
- (ii) For every i there is a $j \neq i$ such that $(C(i), C(j)) \in \mathbb{R}$.
- (iii) $(C(i), C(i)) \notin \mathbb{R}$.

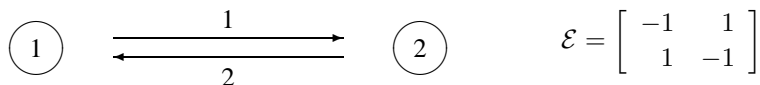
Thus every complex is related to at least one other complex and the trivial reaction $C(i) \rightarrow C(i)$ that produces no change is not admitted. Therefore \mathbb{R} is never reflexive and in general it is neither symmetric nor transitive.

The relation on \mathcal{C} gives rise to a directed graph \mathcal{G} in the following way.² Identify each complex with a vertex V_k in \mathcal{G} and introduce an edge E_ℓ in \mathcal{G} carries a nonnegative weight $P_\ell(c)$ given by the intrinsic rate of the corresponding reaction, and it is assumed throughout that the rate does not vanish identically in \mathbb{R}_n^+ . \mathcal{G} provides a concise representation of the reaction network that clarifies the distinction between the relation \mathbb{R} , which is manifested in the way the vertices are joined by directed edges, and the reaction phenomenology, which is reflected in the weights assigned to the edges.

The topology of \mathcal{G} is in turn represented in its vertex-edge incidence matrix \mathcal{E} , defined as follows.

$$\mathcal{E}_{ij} = \begin{cases} +1 & \text{if } E_j \text{ is incident at } V_i \text{ and is directed toward it} \\ -1 & \text{if } E_j \text{ is incident at } V_i \text{ and is directed away from it} \\ 0 & \text{otherwise} \end{cases}$$

If there are r reactions on \mathcal{C} , then \mathcal{E} has p rows and r columns and every column has exactly one $+1$ and one -1 . For instance, the simple network of two reactions at (2) gives rise to the following graph and incidence matrix.



The rate $P_\ell(C)$ of an elementary reaction $C(i) \rightarrow C(j)$ is generally not a function of $C(i)$, but of the concentration or activity of the individual species in the complex. The rate of a compound reaction can involve all species, and the result is that the temporal evolution of the composition of a reacting mixture is usually not describable in terms of the complexes alone. Nonetheless, one can formally define the rate of change of the complexes as

$$\frac{d}{dt} \begin{pmatrix} C(1) \\ \vdots \\ C(P) \end{pmatrix} = \mathcal{E}P(c), \quad (3)$$

and this is converted into the rate of change of species as follows. Once the complexes and reactions are fixed, the stoichiometry of the complexes is specified unambiguously, and we let ν denote the $n \times p$ matrix whose j^{th} column gives the stoichiometric amounts of the species in the j^{th} complex. Then

$$\frac{dc}{dt} = \nu \frac{d}{dt} \begin{pmatrix} C(1) \\ \vdots \\ C(P) \end{pmatrix} = \nu \mathcal{E}P(c), \quad (4)$$

²The terminology used is standard for the most part; see e.g. Chen (1971), Harary (1969), or Giblin (1977). Several of the less standardized definitions are given later.

and by virtue of the way \mathcal{E} is defined, the columns of the product νE are the stoichiometric vectors of reactions written according to the standard convention (the columns of $\bar{\nu}$). However, the columns of νE are always linearly dependent when reversible reactions are present, because the forward and reverse rates of a reversible pair are considered distinct. In fact, if all reactions are reversible then the rank of νE is no larger than $r/2$.

The rate functions $P_i(c)$ are not completely arbitrary because the solution $\Phi(t, c_0)$ through an initial point c_0 should have the following properties.

- (i) If $c_0 \in \overline{R_n^+}$, $\Phi(t, c_0)$ should exist and be unique for t in some maximal interval $[0, T]$, $T \leq \infty$.
- (ii) If $c_0 \in \overline{R_n^+}$, then $\Phi(t, c_0) \in \overline{R_n^+}$ for $t \in [0, T]$.

Thus (4) should define a local (in t) positive semi-flow for which $\overline{R_n^+}$ is positively invariant. Local existence and uniqueness of solutions will hold if the rate vector $P(c)$ is locally Lipschitzian in c throughout $\overline{R_n^+}$. Nonnegativity of each component of c is guaranteed if the vector field $\nu \mathcal{E} P(c)$ never points out of R_n^+ when the base point lies in ∂R_n^+ (Nagumo 1942), and so the necessary and sufficient condition for (II) is that

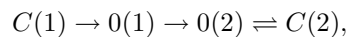
$$\left(\nu \mathcal{E} P(c_1, \dots, \overset{i}{0}, \dots, c_n) \right)_i \geq 0 \quad i = 1, \dots, n \quad (5)$$

for all $c_j \geq 0$, $i \neq j$.

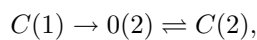
In many systems some of the transport reactions are so rapid that the transported species are always in equilibrium with the bath. Other species, such as water in many biological systems, may be present in such great excess that their concentration changes little even if transport is neglected. As it stands, (4) includes all reacting species, but those whose concentration is constant on the time scale of interest can be ignored. When such a species enters into a reaction its concentration or mole fraction can be absorbed into the rate constant for that reaction and that species can be deleted from each of the complexes in which it appears.³ As a result of these deletions, it will appear that reactions which involve constant species do not necessarily conserve mass. Furthermore, some complexes may not comprise any time-dependent species; these will be called *zero or null complexes*. Each null complex gives rise to a column of zeroes in ν and the rate of any reaction in which the reactant complex is a null complex is usually constant. For instance, any transport reaction of the form $\mathcal{M}^0 \rightarrow \mathcal{M}$ introduces a null complex and the corresponding flux of \mathcal{M} represents a constant input to the reaction network, provided that the rate of the transport step does not depend on the concentration of a time-dependent species. Of course, a constant species that appears in a complex which also contains a variable species likewise represents an input to the network, and to distinguish these from inputs due to null complexes, the former are called *implicit inputs* and the latter are called *explicit inputs*.

Another simplification that can be used to reduce a network is to eliminate fast reactions via singular perturbation arguments. For example, whenever the enzyme-catalyzed conversion $E \rightarrow S \rightleftharpoons ES \rightarrow E + P$ appears, it is often replaced by the step $S \rightarrow P$ where the rate for that step has the Michaelis-Menten form $V_{max} S / (K + S)$. This reduction eliminates one vertex and two edges from the graph and the invariant due to conservation of the enzyme is accounted for by the assumption that $\mathcal{E} \sim \text{constant}$. A rigorous analysis of the validity of such reductions can be found in Heineken et al. (1967).

Finally, if both reactant and product complexes in a reaction are null complexes, that reaction can be eliminated entirely. Thus if $0(1)$ and $0(2)$ are null complexes and the mechanism is



it can be reduced to



thereby eliminating one vertex and one edge.

³Hereafter n will denote the number of species whose concentration may be time-dependent.

The formulation of the dynamical equations given at (4) shows that there are three distinct aspects of a set of reactions that contribute to the over-all rate of change of a species' concentration. These are the stoichiometry of the complexes, as reflected in ν , the underlying structure of the reaction network, which is contained in the incidence matrix \mathcal{E} , and the reaction phenomenology that is embedded in the rate functions $P_i(c)$. In an abstract context, each of these three factors can be varied separately, and our goal is to analyze how each affects the existence of reaction invariants, the structure of the set of time-independent solutions, and the transient behavior of the system. To do this, we must first introduce some more terminology.

3.2 Some Basic Concepts from Graph Theory and Convex Analysis.

Since there is at most one reaction $C(i) \rightarrow C(j)$ for any pair of complexes, a directed edge in \mathcal{G} can be characterized by its initial and terminal vertices and in the following, the ordered pair (i, j) denotes the directed edge from $V_i \rightarrow V_j$. An undirected graph \mathcal{G}^o is obtained from \mathcal{G} by ignoring the orientation of the edges. There are at most two edges connecting any pair of vertices in \mathcal{G}^o , and when it is necessary to distinguish between them they are written $(i, j)_1$ and $(i, j)_2$. Vertices V_i and V_j are said to be *adjacent* if (i, j) is in the edge set of \mathcal{G} , and the *adjacency matrix* \mathcal{A} of \mathcal{G} is defined as follows:

$$\mathcal{A}_{ij} = \begin{cases} +1 & \text{if } (i, j) \text{ is an edge of } \mathcal{G} \\ 0 & \text{otherwise.} \end{cases}$$

An *edge sequence* of length $k - 1$ is a finite sequence of the form $(i_1, i_2)(i_2, i_3) \dots (i_{k-1}, i_k), k \geq 2$. When the edges in an edge sequence are all oriented in the same direction; the sequence is a *directed edge sequence* in \mathcal{G} . When $i_1 = i_k$ the sequence is closed, and otherwise it is open. V_{i_1} is the initial vertex, V_{i_k} is the terminal vertex, and all others are internal vertices. A *path* in \mathcal{G}^o is an open edge sequence in which all vertices are distinct; a *cycle* in \mathcal{G}^o is a closed path in which the internal vertices are distinct. *Directed paths* and *directed cycles* in \mathcal{G} are defined analogously to their counterparts in \mathcal{G}^o , and V_j is said to be *reachable* from V_i if there is a directed path from V_i to V_j . $\mathcal{G}^o(\mathcal{G})$ is said to be *acyclic* if it contains no cycles (directed cycles). The *in-degree* (*out-degree*) of a vertex $V_j \notin \mathcal{G}$ is the number of edges entering (leaving) V_j and these are denoted d_j^+ and d_j^- , respectively. The *degree* d_j of V_j is the sum of the in- and out-degrees.

An undirected graph is *connected* if every pair of vertices is connected by a path. A *component* is a connected subgraph $\mathcal{G}_1 \subseteq \mathcal{G}$ that is maximal with respect to the inclusion of edges, i.e. if \mathcal{G}_2 is a connected subgraph and $\mathcal{G}_1 \subseteq \mathcal{G}_2 \subseteq \mathcal{G}$, then $\mathcal{G}_1 = \mathcal{G}_2$. An isolated vertex is a component and every vertex is contained in one and only one component. A directed graph is *strongly connected* if for every pair $(V_i, V_j), V_i$ is reachable from V_j and vice-versa. A *strongly-connected component* of \mathcal{G} (a *strong component* for short) is a strongly-connected subgraph of \mathcal{G} that is maximal with respect to inclusion of edges. As in the undirected graph, an isolated vertex is a strong component and every vertex belongs to one and only one strong component. It can be shown that a directed graph is strongly connected if and only if there exists a closed, directed edge sequence that contains all the edges in the graph (Chen 1971). Since the union (in a set-theoretic sense) of a directed path from V_i to V_j and a directed path from V_j to V_i is a directed cycle, every strongly connected graph contains at least one directed cycle and the corresponding cycle matrix contains at least one row in which all nonzero entries have the same sign.

An *oriented cycle* in \mathcal{G} is a cycle in \mathcal{G}^o with an orientation assigned by an ordering of the vertices in the cycle. A *cycle matrix* \mathcal{B} associated with \mathcal{G} has elements defined as follows.

$$\mathcal{B}_{ij} = \begin{cases} +1 & \text{if } E_j \text{ is in the } i^{\text{th}} \text{ oriented cycle and the cycle and edge orientation coincide} \\ -1 & \text{if } E_j \text{ is in the } i^{\text{th}} \text{ oriented cycle and the cycle and edge orientation are opposite} \\ 0 & \text{otherwise.} \end{cases}$$

\mathcal{B} is an $r' \times r$ matrix, where r' is the number of independent cycles in \mathcal{G}^o . It has a row in which all nonzero entries have the same sign for every directed cycle in \mathcal{G} .

It proves convenient to associate with \mathcal{G} or \mathcal{G}^o two vector spaces defined as follows. Let $V = \{V_1, \dots, V_p\}$ be the set of vertices of \mathcal{G} and $E = \{E_1, \dots, E_r\}$ the set of edges in \mathcal{G} , and denote by C_0 (C_1) the set of all real-valued functions on V (E). Both C_0 and C_1 have the structure of finite-dimensional real vector spaces, of dimension p and r , respectively. If $f : V \rightarrow R$ then f can be represented by the vector $(v_1, \dots, v_p)^T$, where $v_i = f(V_i)$. The canonical basis $\{b_n | b_j = (0, \dots, 1, \dots, 0), j = 1, \dots, p\}$ in C_0 corresponds to the functions B_j defined by $B_j(V_k) = \delta_{jk}$, where δ_{jk} is the Kronecker delta. An analogous representation holds for functions in C_1 , and both C_0 and C_1 are Euclidean spaces under the standard inner product. Functions in C_0 are called 0-chains and those in C_1 are called 1-chains, although generally the scalars are taken from an Abelian group rather than a field, and C_0 and C_1 are then called chain groups (Hocking and Young 1961; Giblin 1977). Certain aspects of reaction networks have been studied within that framework by Sellers (1966).

In the vector space framework the incidence matrix is the representation with respect to the canonical bases of the 'boundary' operator $\mathcal{E} : C_1 \rightarrow C_0$.⁴ If \mathcal{G} has p vertices and q components then it is easily shown that $\rho(E) = p - q$ (Chen 1971).⁵

Any $e \in \mathcal{N}(\mathcal{E})$ is called a 1-cycle, and 1-cycles are related to the oriented cycles and closed directed edge sequences of \mathcal{G} as follows. For any oriented cycle $\mathcal{G}_1 \in \mathcal{G}$, let $g \in C_1$ be such that

$$g(E_i) = \begin{cases} +1 & \text{if } E_i \in \mathcal{G}_1 \text{ and the orientation of the cycle and the edge coincide} \\ -1 & \text{if } E_i \in \mathcal{G}_1 \text{ and the orientation of the cycle and edge are opposite} \\ 0 & \text{otherwise.} \end{cases}$$

The components of $\mathcal{E}g$ are the inner products of the rows of \mathcal{E} with g , and if $V_j \in \mathcal{G}_1$, the j^{th} row of \mathcal{E} is zero and the corresponding inner product vanishes. If $V_j \in \mathcal{G}_1$ and E_k is incident at V_j , then $\mathcal{E}_{jk} = \pm 1$ according as E_k terminates or originates at V_j , and it is easy to see that the corresponding inner product vanishes. Therefore, if g represents an oriented cycle it is a 1-cycle, and as a result

$$E\mathcal{B}_{(j)}^T = 0 \tag{6}$$

for every row $\mathcal{B}_{(j)}$ of any cycle matrix. This shows that $\mathcal{R}(\mathcal{B}^T) \subseteq \mathcal{N}(E)$ and $\rho(\mathcal{B}) \leq \dim \mathcal{N}(E) = r - p + q$. An elementary argument shows that the rows of \mathcal{B} span $\mathcal{N}(E)$ and therefore

$$r' = \rho(\mathcal{B}) = r - p + q.$$

Any closed directed edge sequence $\mathcal{G}_2 \subset \mathcal{G}$ can be written as the union of directed cycles in \mathcal{G} , and it can be seen that the latter are represented by those 1-cycles in C_1 whose nonzero components are 1. If \mathcal{G} is strongly connected it must contain one or more directed cycles, and it is easy to see that in this case any oriented cycle that is not directed can be written as the symmetric difference of two directed cycles. Consequently, whenever \mathcal{G} is strongly connected, a basis $\{e^i\}$ for $\mathcal{N}(E)$ can be chosen so that $e^i \geq 0$.⁶

A subgraph $T \subseteq \mathcal{G}^o$ is a *tree* if it is connected and acyclic, and a *spanning tree* if it is a tree that contains all the vertices of \mathcal{G}^o . If \mathcal{G}^o is a tree then any two vertices are connected by a unique path and $r = p - 1$. A *cocycle* of \mathcal{G}^o is a minimal set of edges whose removal increases the number of components by one. Every edge of a tree is a cocycle, as is the set of edges incident at a vertex. A cocycle or an edge-disjoint union of cocycles is called a *cutset*, and an *oriented cutset* in \mathcal{G} is a cutset in \mathcal{G}^o with an orientation defined as follows. If V^1 and V^2 are the disjoint subsets into which V is partitioned by a cutset, the orientation of the cutset is specified by ordering the subsets as (V^1, V^2) or as

⁴This operator is usually denoted by ∂ but to simplify notation we use the same symbol for a linear transformation and its representations.

⁵Here and hereafter, $\rho(A)$, $\mathcal{R}(A)$ and $\mathcal{N}(A)$ denote the rank, range and null space of A , respectively. The dimension of a vector space V is denoted $\dim V$.

⁶For any vector u , $u \geq 0$ means that every component is non-negative and at least one is positive, $u \geq 0$ means that all components may be equal to zero, and $u > 0$ means that all components are positive.

(V^2, V^1) . The *cutset matrix* Q of a directed graph \mathcal{G} is the $s' \times p$ matrix obtained by setting

$$Q_{ij} = \begin{cases} 1 & \text{if } E_j \text{ is in cutset } i \text{ and the orientations of the cutset and edge coincide.} \\ -1 & \text{if } E_j \text{ is in cutset } i \text{ and the orientations of the cutset and edge are opposite} \\ 0 & \text{otherwise.} \end{cases}$$

The row dimension of Q is the number of nonempty oriented cutsets, which is

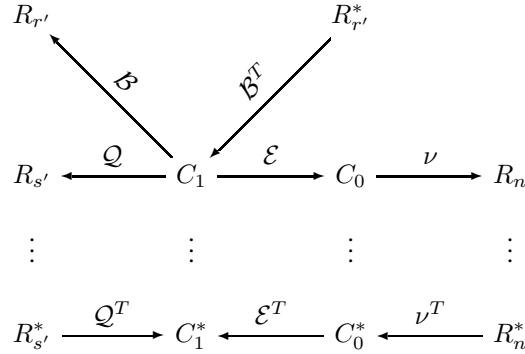
$$\sum_{k=1}^p \binom{p}{k} = 2^p - 1.$$

However, these are not all independent, and in fact it is easy to see that the orientation of the p cutsets that isolate a single vertex can be chosen so that \mathcal{E} is a submatrix of Q . Therefore $\mathcal{R}(Q) \supseteq \mathcal{R}(E)$, and so $\rho(Q) \geq \rho(E)$ but since any cutset can be written in terms of those that isolate a single vertex, equality holds in both cases. It follows from (6) that

$$QB_{(j)}^T = 0 \quad (7)$$

for every row $B_{(j)}$ of any cycle matrix. Hereafter Q will designate a cutset matrix in which the rows are linearly independent, and so s' will equal $p - q$.

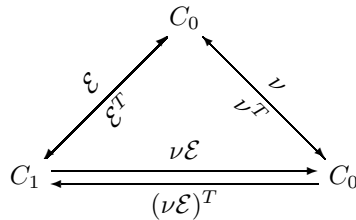
The relationship between the various spaces and maps can be diagrammed as follows.



Here $'^*$ denotes the dual of a space, $R_{r'}$ is the row space of \mathcal{B} and $R_{s'}$ is the row space of \mathcal{M} . Both $R_{s'}$, and $R_{r'}$, can be identified with subspaces of C_1 , of dimension $p - q$ and $r - p + q$, respectively. These are called the cutset and cycle subspaces, respectively, and according to (7) they are orthogonal under the Euclidean inner product. If we identify each space with its dual in the usual way, via isometric isomorphisms defined along the vertical lines of the diagram, then we have the orthogonal decompositions

$$\begin{aligned}
 C_1 &= R(\mathcal{B}^T) \oplus R(Q^T) \\
 &= \mathcal{N}(E) \oplus R(E^T) \\
 C_0 &= R(E) \oplus \mathcal{N}(E^T) \\
 &= \mathcal{N}(\nu) \oplus R(\nu^T) \\
 R_n &= R(\nu) \oplus \mathcal{N}(\nu^T).
 \end{aligned} \quad (8)$$

Of course we can treat $\nu\mathcal{E}$ as a single map from C_1 to R_n , and this viewpoint leads to the following diagram.



It also provides the additional decompositions:

$$C_1 = \mathcal{N}(\nu\mathcal{E}) \oplus R((\nu\mathcal{E})^T)$$

and

$$R_n = R(\nu\mathcal{E}) \oplus \mathcal{N}((\nu\mathcal{E})^T).$$

The usual treatment of kinetics in essence deals only with the horizontal edge of the triangle, but as we shall see, there are cogent reasons for factoring the map νE through C_0 .

A *flow* on \mathcal{G} is a real-valued function on the edge set of \mathcal{G} and it is represented by a vector $f \in C_1$. For a given choice of cycles and cutsets, every flow, or more precisely, its representative, has the unique decomposition into cycles and cutsets given by

$$f = f^0 + f^1 = \mathcal{G}^T w + \mathcal{Q}^T z \quad (9)$$

where $f^0 \in \mathcal{N}(\mathcal{E})$ and $f^1 \in \mathcal{R}(\mathcal{E}^T)$. The vectors w and z are the cycle and cutset weights associated with f . A flow is *balanced* when $z = 0$ ($f^1 = 0$), *cobalanced* when $w = 0$ ($f^0 = 0$), and *positive, nonnegative* or *strictly nonnegative* according as $f > 0$, $f \geq 0$ or $f \geq 0$, respectively. Certain classes of flows treated later are integral flows, which means that the components of f are integers, but we do not require that w and z be integral at present.

The incidence matrix \mathcal{E} is the discrete analog of $-\nabla \cdot$, the negative of the continuum divergence operator, and balanced flows are analogous to solenoidal flows in the fluid-mechanical context.⁷ Indeed, the analogy can be pursued further with a slightly different representation of f , **viz.**

$$f = \mathcal{B}^T w + \mathcal{E}^T \Phi. \quad (10)$$

One may regard $\mathcal{B}^T w$ as the 'curl' of w and $\mathcal{E}^T \Phi$ as the gradient of Φ . The operator $\Delta \equiv -\mathcal{E}\mathcal{E}^T/2$ is the discrete Laplacian, and a balanced flow is one for which

$$2\mathcal{E}f = 0 = -\Delta\Phi.$$

Thus, Φ must be 'harmonic'. The representation at (10) is reminiscent of the Helmholtz decomposition of a vector field \mathbf{v} in three-space into a solenoidal and irrotational part, which yields

$$\mathbf{v} = \nabla \times \mathbf{w} + \nabla\Phi,$$

where $\nabla \cdot \mathbf{w} = 0$ (Aris 1962).

The last series of definitions concerns properties of certain subsets of vector spaces, and for the remainder of this section U denotes an n -dimensional real vector space. A *cone* in U is a closed subset K such that $K \cap \{-K\} = \{0\}$ and $\alpha K + \beta K \subseteq K$ for all real scalars $\alpha, \beta \geq 0$. K is *solid* if its interior is nonempty. A cone is *generated* or *spanned* by a set of vectors in K if any $x \in K$ can be written as a linear combination of vectors in the set, using only nonnegative coefficients. The *dimension* of K is the number of elements in a minimal generating set. A vector $x \in K$ is an *extremal vector* if $x = y + z$ with $y, z \in K$ implies that both y and z are nonnegative multiples of x , and any cone is generated by its extremal vectors (Vandergraft 1968). For any set $S \subseteq U$, the *dual* S^* of S is defined as

$$S^* = \{y \in U \mid \langle x, y \rangle \geq 0 \forall x \in S\}$$

and clearly the dual of a subspace is its orthogonal complement. If K is a cone, the *interior* of K^* is the set

$$\text{Int } K^* = \{y \in K^* \mid x \in K, x \neq 0, \Rightarrow \langle x, y \rangle > 0\}$$

⁷The negative sign arises from our definition of \mathcal{E} , which is the negative of the usual definition, but which is more convenient in the present context. The definition given here makes sense if for every edge (i,j) there is an edge (j,i), but more generally one could define $\Delta = \mathcal{E}\mathcal{E}_o^T$.

and the *boundary* of K^* is the set

$$\partial K^* = \{y \in K^* \mid \exists x \neq 0 \in K \ni \langle x, y \rangle = 0\}.$$

A *face* of a solid cone K is a subset F of K such that F is a cone having the property that if $x - y \in K$, $x \in F$ and $y \in K$, then $y \in F$. For instance, let A be an $m \times n$ matrix. The set of vectors $x \in R_n$ such that $Ax \geq 0$ is a cone in R_n ; it is bounded by the m hyperplanes $\langle A_{(i)}, x \rangle = 0$ where $A_{(i)}$ is the i^{th} row of A . Similarly, the set of vectors $y \in R_m$ such that $y = Ax, x \geq 0$, is a cone in R_m spanned by the n column vectors of A .

4 Reaction Invariants

4.1 General Rate Functions

The decomposition of any flow into cutset and cycle parts means that in the time-dependent equations

$$\frac{dc}{dt} = \nu \mathcal{E} P(c) \quad (11)$$

we can write

$$P(c) = P_1(c) + P_2(c),$$

where $P_1 \in \mathcal{N}(\mathcal{E})$ and $P_2 \in R(\mathcal{E}^T)$. Consequently,

$$\frac{dc}{dt} = \nu \mathcal{E} P_2(c)$$

and so only the cutset part of any flow enters the transient equations. Said otherwise, if the flow is balanced at some instant t_0 then $dc/dt = 0$ for all $t \geq t_0$, and as a result, a time-dependent flow cannot be balanced. Moreover, if $\mathcal{E} P_2(c) \in \mathcal{N}(\nu)$, $c(t)$ must again be constant, and to further analyze the transient and steady state behavior of (11) we must analyze $\mathcal{R}(\nu \mathcal{E})$ in more detail.

Every elementary chemical reaction in the network conserves mass, although this may not be apparent after the concentration of each time-invariant species is absorbed into a rate constant. However, the total mass of the mixture need not be constant because the system is open and there may be no quantities that are conserved during reaction. A vector $\Omega \in R_n$ defines an invariant linear combination of concentrations if

$$\langle \Omega, \nu \mathcal{E} P(c) \rangle = 0, \quad (12)$$

for then

$$\langle \Omega, \frac{dc}{dt} \rangle = 0$$

and so

$$\langle \Omega, c(t) \rangle = \langle \Omega, c(0) \rangle. \quad (13)$$

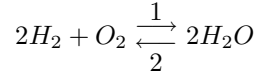
The solutions Ω of (12), which we shall call invariants when no confusion can arise, span three disjoint subspaces $I_j \subset R_n$, of respective dimension i_j , defined as follows.

$$\begin{aligned} I_1 &\equiv \mathcal{N}(\nu^T) \\ I_2 &\equiv \text{span}\{\Omega \in R_n \mid \nu^T \Omega \in \mathcal{N}(\mathcal{E}^T), \langle \Omega, z \rangle = 0 \forall z \in I_1\} \\ I_3 &\equiv \text{span}\{\Omega \in R_n \mid \langle \mathcal{E}^T \nu^T \Omega, P(c) \rangle = 0 \forall c \in \overline{R_n^+}, \mathcal{E}^T \nu^T \Omega \neq 0\}. \end{aligned}$$

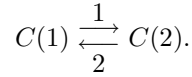
According to (13), each invariant Ω can be thought of as comprising the 'stoichiometric' coefficients of a non-reacting complex, although in general the coefficients are not integral and some may be negative. Those in I_1 are independent

of both the network structure and the rate functions; they are fixed solely by the stoichiometry of the complexes. In some networks the non-null complexes are all species or multiples of species and in such cases $i_1 = 0$, for then $\nu = [\nu_1 \mid 0]$, where ν_1 is an $n \times n$ diagonal matrix. A similar conclusion holds when the species and complexes can be ordered so that ν_1 is either lower or upper triangular.

The existence of an $\Omega \in I_1$ indicates that the n species are not all required to define the stoichiometry of the complexes, but since $\nu_{ij} \geq 0$, there are no $\Omega \geq 0$ in I_1 . Therefore such Ω 's indicate that certain differences of species concentrations are conserved. For example, consider the reaction



which is represented as



Here

$$\nu = \begin{bmatrix} 2 & 0 \\ 1 & 0 \\ 0 & 2 \end{bmatrix} \quad \mathcal{E} = \begin{bmatrix} -1 & 1 \\ 1 & -1 \end{bmatrix}$$

$$\mathcal{N}(\nu^T) = \text{span}\{(1, -2, 0)^T\} \equiv \Omega_1$$

and so the invariant combination of concentrations is $c_1(t) - 2c_2(t)$.

Clearly $I_2 \subseteq \mathcal{R}(\nu)$, but more precisely,

$$I_2 = \text{preimage}[\mathcal{R}(\nu^T) \cap \mathcal{N}(\mathcal{E}^T)].$$

Therefore

$$i_2 = \dim[\mathcal{R}(\nu^T) \cap \mathcal{N}(\mathcal{E}^T)] \leq \min\{n - i_1, q\}$$

and there can be no more independent invariants in I_2 than there are components in \mathcal{G} . In particular, if \mathcal{G} is connected then $i_2 \leq 1$, and if there is a null complex as well, then $i_2 = 0$.

It is easily seen that the complexes can be labeled so that any $u \in \mathcal{N}(\mathcal{E}^T)$ has the form

$$u = \bigoplus_{\alpha=1}^q \omega_\alpha u_\alpha \tag{14}$$

where u_α is a p_α -dimensional vector of ones, the ω_α 's are scalars, and p_α is the number of vertices in the α^{th} component of G . Consequently, $i_2 \neq 0$ if and only if $\nu^T \Omega = u$ has a solution, i.e., if and only if $\langle v, u \rangle = 0$ for every $v \in \mathcal{N}(\nu)$. When the complexes are linearly independent, ν^T has a right inverse and i_2 is certainly non-zero. This is the case in the previous example, and one finds that a solution of

$$\begin{bmatrix} 2 & 1 & 0 \\ 0 & 0 & 2 \end{bmatrix} \begin{pmatrix} \Omega_{12} \\ \Omega_{22} \\ \Omega_{32} \end{pmatrix} = \begin{pmatrix} 1 \\ 1 \end{pmatrix}$$

is $\Omega_2 = (2/5, 1/5, 1/2)^T$. Taken together, Ω_1 and Ω_2 span $\mathcal{N}(\mathcal{E}^T \nu^T)$, and the reader can readily show that the invariants that represent conservation of H and O atoms can be constructed from Ω_1 and Ω_2 .

The invariants in I_1 and I_2 will be called kinematic invariants because their existence does not depend on the rate functions $P_i(c)$. Since $\mathcal{N}(\mathcal{E}^T \nu^T) = I_1 \oplus I_2$, the number of independent reactions in the network, call it s , is $n - (i_1 + i_2)$. The orthogonal complement $\mathcal{R}(\nu \mathcal{E})$ of $\mathcal{N}(\mathcal{E}^T \nu^T)$ is called the reaction subspace, or more properly, the

kinematic subspace defined by the mechanism. The intersection of the coset of $\mathcal{R}(\nu\mathcal{E})$ through a point $c_0 \in \overline{R_n^+}$ with $\overline{R_n^+}$ is a closed subset of \mathcal{R}_n called the reaction simplex through c_0 . We denote this by $\Omega(c_0)$. It is a simplex in the mathematical sense when it is bounded, and hence compact. The decomposition of \mathcal{R}_3 of the preceding example is shown in Figure 1.

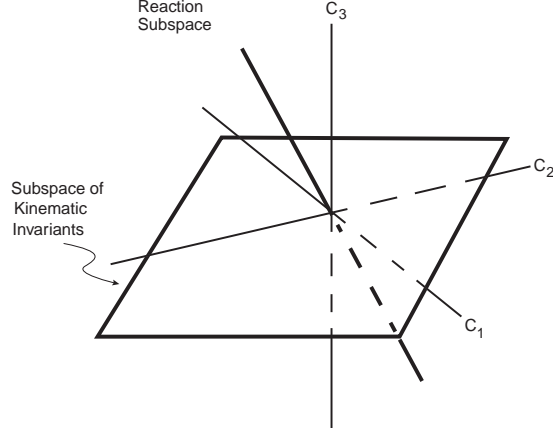


Figure 1: The decomposition of state space into a subspace of kinematic invariants and a complement, the reaction subspace

The number of kinematic invariants is related to other indices of the network in the following way. Since

$$\mathcal{R}(\nu^T) + \mathcal{N}(\mathcal{E}^T) = \mathcal{N}(\nu)^\perp + \mathcal{R}(\mathcal{E})^\perp = [\mathcal{N}(\nu) \cap \mathcal{R}(\mathcal{E})]^\perp \quad (15)$$

it follows that

$$\dim \mathcal{R}(\nu^T) + \dim \mathcal{N}(\mathcal{E}^T) - \dim[\mathcal{R}(\nu^T) \cap \mathcal{N}(\mathcal{E}^T)] = \dim[\mathcal{N}(\nu) \cap \mathcal{R}(\mathcal{E})]^\perp$$

and so

$$n - i_1 + q - i_2 = p - \dim[\mathcal{N}(\nu) \cap \mathcal{R}(\mathcal{E})].$$

Let $\delta = \dim[\mathcal{N}(\nu) \cap \mathcal{R}(\mathcal{E})]$; then

$$\delta = p - q - (n - (i_1 + i_2)) = p - 1 - s = \rho(E) - \rho(\nu\mathcal{E}). \quad (16)$$

Thus δ is the difference between the maximal number of independent reactions based on the structure of the graph and the actual number of independent reactions. This number, which is clearly non-negative, is called the deficiency by (?), and when it vanishes ν does not annihilate any elements in $\mathcal{R}(\mathcal{E})$. When $\delta = 0$, ν is one-to-one from $\mathcal{R}(\mathcal{E})$ to $\mathcal{R}(\nu\mathcal{E})$ and therefore has a left inverse \overline{N} from $\mathcal{R}(\nu\mathcal{E})$ to $\mathcal{R}(\mathcal{E})$. In this case the reaction subspace is isomorphic to $\mathcal{R}(\mathcal{E})$ and the natural definition of the complex concentrations is $\overline{C} = \overline{N}c$, for then

$$\frac{d\overline{C}}{dt} = \overline{N} \frac{dc}{dt} = \mathcal{E}P(c),$$

as in the formal definition at (3). Because $\mathcal{R}(\mathcal{E})$ and $\mathcal{R}(\nu\mathcal{E})$ are isomorphic the dynamical behavior can be described in terms of complexes only, simply by setting $c(t) - c(0) = \nu[\overline{C}(t) - \overline{C}(0)]$. However, it should be noted that these definitions may lead to negative complex concentrations, and that only $p - q$ independent complexes are needed as coordinates in $\mathcal{R}(\mathcal{E})$.

The dimension of the third subspace of invariants, I_3 , can be determined as follows. Any $\Omega \in I_3$ can be written

$$\Omega = \Omega_1 + \Omega_2$$

where $\Omega_1 \in \mathcal{N}(\mathcal{E}^T \nu^T)$ and $\Omega_2 \in R(\nu \mathcal{E})$. Therefore

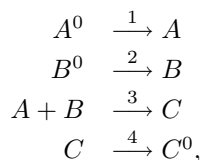
$$\langle \mathcal{E}^T \nu^T \Omega, P(c) \rangle = \langle \Omega_2, \nu \mathcal{E} P(c) \rangle$$

as before, and so it is necessary that either $P_2(c) \equiv 0$, in which case $P(c)$ is identically proportional to an oriented cycle, or the cutset part must satisfy

$$\langle \Omega_2, \nu \mathcal{E} P_2(c) \rangle = 0.$$

The latter requires that either $\Omega_2 = 0$, which means that $\Omega \notin I_3$, or $\nu \mathcal{E} P_2(c)$ must vanish identically. Consequently, i_3 is certainly zero if $\delta = 0$ and \mathcal{G} is acyclic, and if $\delta > 0$, $i_3 > 0$ only if the cutset part is such that $\mathcal{E} P_2(c) \in \mathcal{N}(\nu)$ for all $c \in \overline{R_n^+}$. Therefore $i_3 \leq \delta$ whenever it is positive, and $dc/dt \equiv 0$ in this case. Obviously this is a very degenerate situation, as the following example illustrates.

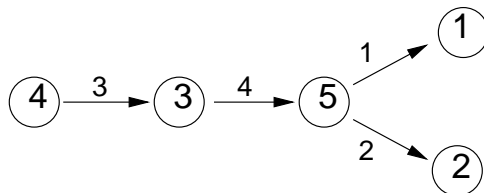
Suppose that the mechanism is



where A^0 , B^0 and C^0 are held constant. We order the active species in alphabetical order, identify A^0 , B^0 and C^0 with $C(5)$, and label the remaining complexes as

$$\begin{array}{l} C(1) = A \\ C(2) = B \\ C(3) = C \\ C(4) = A + B. \end{array}$$

The graph \mathcal{G} is



and so $(p, q, r) = (5, 1, 4)$. One finds that

$$\nu = \begin{bmatrix} 1 & 0 & 0 & 1 & 0 \\ 0 & 1 & 0 & 1 & 0 \\ 0 & 0 & 1 & 0 & 0 \end{bmatrix} \quad \mathcal{E} = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & -1 \\ 0 & 0 & -1 & 0 \\ -1 & -1 & 0 & 1 \end{bmatrix}$$

and it follows that $\rho(\mathcal{E}) = 4$, $\rho(\nu \mathcal{E}) = 3$, and $i_1 = i_2 = 0$. Since \mathcal{G} is a tree, $P_1(c) = 0$, and some computation shows that $i_3 = 1$ if and only if $P = P_2$ has the form

$$P(c) = \lambda(c) \begin{pmatrix} 1 \\ 1 \\ 1 \\ 1 \end{pmatrix}$$

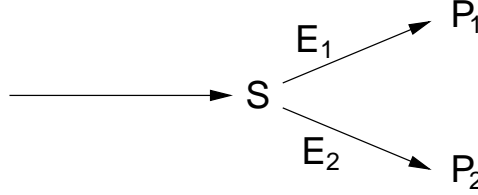
where $\lambda(c) \neq 0$. Thus if all the rates are the same function of c , every point in $\overline{R_n^+}$ is a steady state composition. Needless to say, it is very rare that $i_3 > 0$.

4.2 Mass Action and Related Types of Rate Functions.

A special but important class of rate functions is that in which the rate of the i^{th} reaction can be written

$$P_i(c) = k_{ij}R_j(c) \quad \begin{array}{l} i = 1, \dots, r \\ j = 1, \dots, p \end{array} \quad (17)$$

for every reaction that involves the j^{th} complex as the reactant. This includes ideal mass action rate laws, in which the rate is proportional to the product of the concentrations of the species in the reactant complex, each concentration raised to a power equal to the stoichiometric coefficient of the corresponding species in the complex. It also includes enzyme-catalyzed pathways of the form



provided that the Michaelis constants for the enzymes E_1 and E_2 are identical.

The rate vector $P(c)$ can be written

$$P(c) = KR(c) \quad (18)$$

where K is an $r \times p$ matrix with $k_{ij} > 0$ if and only if the i^{th} edge leaves the j^{th} vertex, and $k_{ij} = 0$ otherwise. The topology of the underlying graph \mathcal{G} enters into K as follows. Define the *entrance matrix* \mathcal{E}_i of \mathcal{G} by replacing all -1 's in \mathcal{E} by zero, and define the exit matrix \mathcal{E}_0 as $\mathcal{E}_i - \mathcal{E}$. Let \hat{K} be the $r \times r$ diagonal matrix with the k_{ij} 's, $i = 1, \dots, r$, along the diagonal, ordered by their first subscript. Then it is easy to see that $K = \hat{K}\mathcal{E}_0^T$ and that

$$\mathcal{E}K = \mathcal{E}\hat{K}\mathcal{E}_0^T = \mathcal{E}_i\hat{K}_0^T - \mathcal{E}_0\hat{K}\mathcal{E}_0^T. \quad (19)$$

It follows from the definitions of \mathcal{E}_0 and \mathcal{E}_i that

- (i) The $(p, q)^{\text{th}}$ entry of $\mathcal{E}_i\hat{K}\mathcal{E}_0^T$ is nonzero (and positive) if and only if there is a directed edge $(q, p) \in \mathcal{G}$.
- (ii) $\mathcal{E}_0\hat{K}_0^T$ is a $p \times p$ diagonal matrix whose j^{th} entry is the sum of the k_{ij} 's for all edges that leave the j^{th} vertex.
- (iii) The columns of $\mathcal{E}\hat{K}_0^T$ all sum to zero, and so $\rho(\mathcal{E}\hat{K}_0^T) \leq p - 1$.

Since \mathcal{E}_0 is fixed by the network, it is advantageous to modify the definitions of I_2 and I_3 slightly. We define

$$I_2 \equiv \text{span}\{\Omega \in \mathbb{R}_n \mid \nu^T \Omega \in \mathcal{N}(\mathcal{E}_0\hat{K}\mathcal{E}^T), \langle \Omega, z \rangle = 0 \forall z \in \mathcal{N}(\nu^T)\}$$

and alter the definition of I_3 in the obvious way. The advantage of modifying these definitions lies in the fact that now $I_3 = \{0\}$ for mass action kinetics, either of ideal or non-ideal type, provided only that the activity $a_1 \rightarrow 0$ as $c_i \rightarrow 0$ in the latter case. As a result, all invariants are kinematic invariants in the extended sense.

The maximum number of independent kinematic invariants in i_2 is $\dim \mathcal{N}(\mathcal{E}_0\hat{K}\mathcal{E}^T)$, and this number can be determined as follows. Suppose that \mathcal{G} has q components \mathcal{G}_α , $\alpha = 1, \dots, q$, and that there are p_α vertices in \mathcal{G}_α . For each \mathcal{G}_α determine the strong components $\mathcal{G}_{\alpha\beta}$ and partition the vertex set to conform with this partition of the graph. Thus write

$$V = \{V_1, V_2, \dots, V_q\}$$

and

$$V_\alpha = \{V_{\alpha 1}, \dots, V_{\alpha \gamma(\alpha)}\}^8$$

⁸Any or all of the $V_{\alpha\beta}$ may consist of single vertices.

where $\gamma(\alpha)$ is the number of strong components in \mathcal{G}_α . The V_j are disjoint and the edge set can be partitioned in the same way as V ; thus $E\hat{K}E_0^T$ has the direct sum decomposition

$$E\hat{K}E_0^T = \oplus_\alpha \mathcal{E}^\alpha \hat{K}^\alpha (\mathcal{E}_0^\alpha)^T$$

where E^α is the incidence matrix for \mathcal{G}_α . The partition of \mathcal{G} into components leads to the decompositions

$$\begin{aligned} C_0 &= \oplus_\alpha C_{0\alpha} \\ C_1 &= \oplus_\alpha C_{1\alpha} \end{aligned} \tag{20}$$

where $C_{0\alpha}$ has dimension p_α and $C_{1\alpha}$ has dimension r_α . It follows that we need only consider $\rho(\mathcal{E}^\alpha \hat{K}^\alpha \mathcal{E}_0^{\alpha T})$ for a fixed α , for the results will be additive in α . For simplicity we suppress the α on \mathcal{E} and \hat{K} until further notice.

The strong components $\mathcal{G}_{\alpha\beta}$ in the partition of \mathcal{G}_α are of three types, namely,

- (i) those in which no edges from other strong components terminate; such strong components are called *sources*
- (ii) strong components on which edges from other strong components terminate and from which edges to other strong components originate; these are called *internal* strong components
- (iii) those from which no edges to other strong components originate; these are called *absorbing* strong components or *sinks*.

Clearly no vertex in a source is reachable from any vertex outside its component and no vertex in a sink is reachable from a vertex in any other sink. Thus the relationship of reachability defines a partial order on the strong components of \mathcal{G}_α , and this in turn leads to the acyclic skeleton $\overset{\circ}{\mathcal{G}}_\alpha$ of \mathcal{G}_α , which is defined as follows. Associate a vertex $\overset{\circ}{V}_j$ with each strong component of \mathcal{G}_α , and introduce a directed edge from $\overset{\circ}{V}_i$ to $\overset{\circ}{V}_j$ if and only if one (and hence every) vertex in $V_{\alpha j}$ is reachable from $V_{\alpha i}$. $\overset{\circ}{\mathcal{G}}_\alpha$ is connected since \mathcal{G}_α is connected, but it is acyclic; in fact, it is a directed tree. Either $\overset{\circ}{\mathcal{G}}_\alpha$ consists of a single vertex and no edges, which occurs when \mathcal{G}_α consists of one strong component, or it has at least one vertex of in-degree zero and at least one vertex of out-degree zero. By relabeling the strong components if necessary, the adjacency matrix of $\overset{\circ}{\mathcal{G}}_\alpha$ can be put into the form

$$\mathcal{A} = \left[\begin{array}{cc|cc|cc} 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 \\ \hline x & x & 0 & 0 & 0 & 0 \\ x & x & x & 0 & 0 & 0 \\ \hline x & x & x & x & 0 & 0 \\ x & x & x & x & 0 & 0 \end{array} \right]$$

where the x 's represent blocks that may be non-zero. The three diagonal blocks are square matrices of dimensions equal to the number of sources, the number of internal strong components, and the number of sinks, respectively. Note that the vertices corresponding to internal strong components can always be ordered so that the central block is lower triangular by virtue of the fact that the strong components are maximal with respect to inclusion of edges. The number of sinks or absorbing strong components is the number of zero columns of \mathcal{A} .

The edge set of \mathcal{G}_α can be partitioned into γ_α subsets $\mathcal{E}_{\alpha j}$, the j^{th} of which contains all the edges that leave the

vertices in the j^{th} strong component, and the incidence matrix can then be written as follows.

$$\mathcal{E} = \begin{bmatrix} \mathcal{E}_{11} & 0 & 0 & 0 & 0 & 0 \\ 0 & \mathcal{E}_{22} & 0 & 0 & 0 & 0 \\ \mathcal{E}_{31} & \mathcal{E}_{32} & \mathcal{E}_{33} & \cdots & & 0 \\ \mathcal{E}_{41} & \mathcal{E}_{42} & \mathcal{E}_{43} & \mathcal{E}_{44} & \cdots & 0 \\ \mathcal{E}_{51} & \mathcal{E}_{52} & \mathcal{E}_{53} & \mathcal{E}_{54} & \mathcal{E}_{55} & 0 \\ \mathcal{E}_{61} & \mathcal{E}_{62} & \mathcal{E}_{63} & \mathcal{E}_{64} & 0 & \mathcal{E}_{660} \end{bmatrix} \quad (21)$$

(For illustrative purposes, we have written \mathcal{E} for a case in which there are two each of sources, sinks and internal strong components.) The non-zero elements in the off-diagonal blocks are all $+1$, and since \mathcal{E}_0 is formally obtained from \mathcal{E} by dropping $+1$'s and changing the sign of -1 's, it follows that

$$\mathcal{E}_0 = \begin{bmatrix} \mathcal{E}_{110} & 0 & 0 & 0 & 0 & 0 \\ 0 & \mathcal{E}_{220} & \cdots & & & 0 \\ 0 & 0 & \mathcal{E}_{330} & \cdots & & 0 \\ 0 & 0 & 0 & \mathcal{E}_{440} & \cdots & 0 \\ 0 & 0 & 0 & 0 & \mathcal{E}_{550} & 0 \\ 0 & 0 & 0 & 0 & 0 & \mathcal{E}_{660} \end{bmatrix} \quad (22)$$

If \hat{K} and x are partitioned in conformance with \mathcal{E} , then the system

$$\mathcal{E}\hat{K}\mathcal{E}_0^T x = 0$$

can be written in the block form

$$\begin{bmatrix} \mathcal{E}_{11}\hat{K}_1\mathcal{E}_{110}^T & 0 & 0 & 0 & 0 & 0 \\ 0 & \mathcal{E}_{22}\hat{K}_2\mathcal{E}_{220}^T & 0 & 0 & 0 & 0 \\ \mathcal{E}_{31}\hat{K}_1\mathcal{E}_{110}^T & \vdots & \mathcal{E}_{33}\hat{K}_3\mathcal{E}_{330}^T & 0 & 0 & 0 \\ \vdots & & \mathcal{E}_{43}\hat{K}_3\mathcal{E}_{330}^T & \mathcal{E}_{44}\hat{K}_4\mathcal{E}_{440}^T & 0 & 0 \\ \vdots & & & & \mathcal{E}_{55}\hat{K}_5\mathcal{E}_{550}^T & 0 \\ \mathcal{E}_{61}\hat{K}_1\mathcal{E}_{110}^T & \cdots & \cdot & & 0 & \mathcal{E}_{66}\hat{K}_6\mathcal{E}_{660}^T \end{bmatrix} \begin{pmatrix} x_1 \\ x_2 \\ x_3 \\ x_4 \\ x_5 \\ x_6 \end{pmatrix} = 0.$$

Therefore the first step in finding $\dim \mathcal{N}(\mathcal{E}_0\hat{K}\mathcal{E}^T)$ is to determine the rank of each of the diagonal blocks.

The diagonal blocks \mathcal{E}_{jj} of \mathcal{E} are not the incidence matrices of a subgraph but they can be decomposed as

$$\mathcal{E}_{jj} = [\mathcal{E}_{j1} \mid \mathcal{E}_{j2}],$$

where \mathcal{E}_{j1} is the incidence matrix corresponding to all edges that originate and terminate within $\mathcal{G}_{\alpha j}$, while \mathcal{E}_{j2} corresponds to edges that originate in $\mathcal{G}_{\alpha j}$ but terminate in another strong component of \mathcal{G}_{α} . Similarly,

$$\mathcal{E}_{jj0} = [\mathcal{E}_{j10} \mid -\mathcal{E}_{j2}]$$

and so

$$\mathcal{E}_{jj}\hat{K}_j\mathcal{E}_{jj0}^T = \mathcal{E}_{j1}\hat{K}_j - j\mathcal{E}_{j10}^T - \mathcal{E}_{j2}\hat{K}_j\mathcal{E}_{j2}^T.$$

For every absorbing strong component $\mathcal{E}_{j2}\hat{K}_j\mathcal{E}_{j2}^T$ is absent, and consequently, $\mathcal{E}_{j1}\hat{K}_j\mathcal{E}_{j10}^T$ corresponds to a strongly-connected subgraph consisting of the edges that originate and terminate within the component. The rank of $\mathcal{E}_{j1}\hat{K}_j\mathcal{E}_{j10}^T$ for such components is one less than the number of vertices in the component, as is shown in the following proposition.

Proposition 1 If \mathcal{G}_α is strongly connected, then $\rho(\mathcal{E}\hat{K}\mathcal{E}_0^T) = p_\alpha - 1$.

Proof. : From properties (i) and (ii) of $\mathcal{E}\hat{K}\mathcal{E}_0^T$ given earlier, it follows that for s sufficiently large, $A(s) \equiv \mathcal{E}\hat{K}\mathcal{E}_0^T + sI$ is non-negative. Since \mathcal{G}_α is strongly connected, $\mathcal{E}\hat{K}\mathcal{E}_0^T$ is irreducible (cf. (Berman and Plemmons 1979)) and so is $A(s)$. Therefore the Perron root $r(A)$ is such that

$$\min_j \sum_i A_{ij} \leq \max_j \sum_i A_{ij}$$

with equality on either side if and only if the minimum and maximum sums are equal (Seneta 1973; Berman and Plemmons 1979). Since these sums are s , $r(A) = s$ and it follows that $\mathcal{E}\hat{K}\mathcal{E}_0^T$ has a simple zero eigenvalue; **i.e.**, $\rho(\mathcal{E}\hat{K}\mathcal{E}_0^T) = p_\alpha - 1$.

We know that $\mathcal{E}^T u = 0$ where $u = (1, \dots, 1)^T$, and therefore the left eigenvector of $\mathcal{E}\hat{K}\mathcal{E}_0^T$ associated with the zero eigenvalue is u .

The foregoing shows that for any \mathcal{G}_α ,

$$\rho(\mathcal{E}\hat{K}\mathcal{E}_0^T) \leq p - (\# \text{ of absorbing strong components}).$$

In fact this is an equality, as is shown next. For every non-absorbing strong component, $\mathcal{E}_{j_2}\hat{K}_j\mathcal{E}_{j_2}^T$ is a diagonal matrix with non-negative diagonal elements, and therefore

$$B(s) \equiv \mathcal{E}_{j_1}\hat{K}_j\mathcal{E}_{j_0}^T - \mathcal{E}_{j_2}\hat{K}_j\mathcal{E}_{j_2}^T + sI$$

is non-negative for sufficiently large s . Since \mathcal{E}_{j_1} is the incidence matrix of a strong component, $B(s)$ is irreducible and the Perron root is again bounded between the maximum and minimum column sums. Since at least one edge leaves every non-absorbing strong component, there exists an $\varepsilon > 0$ such that

$$s - \varepsilon \leq r(B) \leq s.$$

Therefore the spectrum of $\mathcal{E}_{j_1}\hat{K}_j\mathcal{E}_{j_0}^T - \mathcal{E}_{j_2}\hat{K}_j\mathcal{E}_{j_2}^T$ lies strictly within the left-half plane, and so the diagonal blocks corresponding to non-absorbing strong components are all non-singular. Consequently, for every $x \in \mathcal{N}(\mathcal{E}\hat{K}\mathcal{E}_0^T)$, $x_i = 0$ if the i^{th} component is non-absorbing, which proves that $\dim \mathcal{N}(\mathcal{E}\hat{K}\mathcal{E}_0^T)$ is equal to the number of absorbing strong components in \mathcal{G}_α . By adding the results over all components of \mathcal{G} one obtains the following theorem.

Theorem 2 Let \mathcal{G} be a graph with q components \mathcal{G}_α , and let N_α be the number of absorbing strong components in \mathcal{G}_α . Then⁹

$$N \equiv \dim \mathcal{N}(\mathcal{E}\hat{K}\mathcal{E}_0^T) = \dim \mathcal{N}(\mathcal{E}_0\hat{K}E^T) = \sum_{\alpha=1}^q N_\alpha. \quad (23)$$

The theorem provides an upper bound for i_2 but the actual number can only be determined after the stoichiometry is specified. It should be noted that because the number of strong absorbing components of \mathcal{G} is at least q , $N \geq q = \dim \mathcal{N}(\mathcal{E}^T)$. Consequently, if any \mathcal{G}_α has more than one absorbing strong component then it can happen that $i_2 > \dim \mathcal{N}(\mathcal{E}^T)$, which would indicate that the subspace I_2 for mass-action-type rate functions contains invariants that would appear in I_3 if the definitions of the preceding section were applied.

⁹Here \mathcal{E} and \hat{K} refer to the entire graph \mathcal{G}

4.3 Compactness of the Reaction Simplex.

In closed systems the total mass of the mixture is conserved, and as a result, there is an $\Omega > 0$ in $\mathcal{N}(\mathcal{E}^T \nu^T)$. This in turn implies that $\Omega(c_0)$ is bounded, and hence compact, and an application of Brouwer's fixed point theorem shows that there is at least one equilibrium point (Wei 1962). A similar conclusion holds for open systems when a positive Ω exists, as the following proposition due to Horn and Jackson (1972) shows. To avoid the trivial situation in which every $c \in \overline{R_n^+}$ is a steady state, we assume hereafter that $i_3 = 0$.

Proposition 3 Let $0 < c_0 < \infty$ be given. Then $\Omega(c_0)$ is bounded, and hence compact, if and only if there is an $\Omega > 0$ in $\mathcal{N}(\mathcal{E}^T \nu^T)$.

Proof. : Suppose that there is an $\Omega > 0$ in $\mathcal{N}(\mathcal{E}^T \nu^T)$. Since the components of Ω are finite,

$$\langle \Omega, c \rangle = \langle \Omega, c_0 \rangle < \infty,$$

and the intersection of this hyperplane with $\overline{R_n^+}$ is necessarily a bounded set. Conversely, suppose that $\Omega(c_0)$ is bounded. Since

$$\Omega(c_0) = \{c_0 + \mathcal{R}(\nu\mathcal{E})\} \cap \overline{R_n^+}$$

it follows that

$$\mathcal{R}(\nu\mathcal{E}) \cap \overline{R_n^+} = \{0\}$$

because $\Omega(c_0)$ is bounded. The existence of an $\Omega > 0$ is now a direct consequence of an alternative theorem due to Stiemke (1915), which asserts that either the system

$$\mathcal{E}^T \nu^T \Omega = 0 \tag{24}$$

has a solution $\Omega > 0$ or the system

$$\nu\mathcal{E}z \geq 0 \tag{25}$$

has a solution z , but never both. As was noted earlier, there is no $\Omega \geq 0$ in I_1 , and therefore $\Omega(c_0)$ is compact if and only if

$$\nu^T \Omega = u \tag{26}$$

has a positive solution. Here u is given by (14), in which the scalars ω_j are now non-negative. It is permissible that some $\omega_j = 0$, but only if the corresponding columns of ν^T are zero. However, the latter means that some species do not appear in any complexes and without loss of generality they can be ignored. Therefore we require that $\omega_j > 0$ for all j .

Let U be the $p \times q$ matrix given by

$$U = \begin{bmatrix} u_1 & 0 & \cdot & \cdot & \cdot & 0 \\ 0 & u_2 & & & & \cdot \\ \cdot & 0 & & & & \cdot \\ \cdot & \cdot & & & & \cdot \\ \cdot & \cdot & & & & \cdot \\ \cdot & \cdot & & & & \cdot \\ 0 & 0 & & & & u_q \end{bmatrix}$$

Then the problem of solving (25) is equivalent to finding a solution $(\Omega, \omega) > (0, 0)$ of the system

$$[\nu^T \mid -U] \begin{pmatrix} \Omega \\ \omega \end{pmatrix} = 0. \tag{27}$$

By Stiemke's theorem, this has a positive solution if and only if the system

$$\begin{bmatrix} \nu \\ -U^T \end{bmatrix} x \geq 0 \quad (28)$$

has no solution.

Proposition 4 If there is at least one null complex in the network, then (26) (or (27)) does not have a positive solution.

Proof. : First suppose that $q = 1$ and that there are p^1 null complexes. Write

$$\nu = [\nu_1 \mid 0]$$

where ν_1 is $n \times (p-p^1)$, and partition x to conform with the partition of ν . Then (28) reads

$$\begin{aligned} \nu_1 x_1 &\geq 0 \\ \sum_{i=1}^{p-p^1} x_{1i} + \sum_{j=1}^{p^1} x_{2j} &\leq 0. \end{aligned}$$

The first of these is satisfied if we choose $x = (1, 1 \dots 1)^T$ and the second can be satisfied by an appropriate choice of x_2 . Therefore (27) has a solution and so (26) has no positive solution.

When $q > 1$, suppose that there is exactly one null complex, and by relabeling if necessary, suppose that it appears in the first component. Partition the vertex set of \mathcal{G} as in Section 3.2 and partition ν to conform with the partition. Then

$$\nu = [[\nu_1 \mid 0] \mid \nu_2 \mid \dots \mid \nu_q]$$

and given a conformal partition of x , (28) becomes

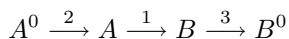
$$\nu_1 x_{11} + \sum_{j=2}^q \nu_j x_j \geq 0 \quad (29)$$

$$\sum_{j=1}^{p_1-p^1} (x_{11})_j + \sum_{k=1}^{p^1} (x_{12})_k \leq 0 \quad (30)$$

$$\sum_{k=1}^{p_\alpha} x_{\alpha k} \leq 0 \quad \alpha = 2, \dots, q. \quad (31)$$

If we choose $x_\alpha = 0$, $\alpha = 2, \dots, q$, then (31) is satisfied and (29) and (30) are identical to the equations for $q = 1$. This proves the proposition when there is only one null complex, and an analogous argument covers the general case.¹⁰

The consequence of this result is that when there are null complexes in the network one cannot assert *a priori* that a positive steady state exists. The following example illustrates why one cannot expect to do better using stoichiometric information alone. Suppose that the reaction network is



¹⁰As will be shown later, a network with more than one null complex can always be transformed into an equivalent network with one null complex.

and that reaction 1 is enzyme-catalyzed and follows Michaelis-Menten kinetics. If the rate of the input reaction is constant and exceeds the V_{\max} of the enzyme, the concentration of A will simply increase monotonically and there will be no steady state.

Another class of mechanisms for which (25) has no positive solution is given by the following proposition. Without loss of generality we assume that there are no null complexes in the network.

Proposition 5 Suppose that the complexes of a network are distinct and that the stoichiometric vectors of two complexes in the same component of \mathcal{G} are proportional. Then there is no $\Omega > 0$ in $\mathcal{N}(\mathcal{E}^T \nu^T)$.

The proof is left to the reader. It is not true that proportionality of complexes in different components precludes the existence of an $\Omega > 0$, a fact that is demonstrated by a mechanism due to (Wegscheider 1902).



It is easily shown that $\mathcal{N}(\mathcal{E}^T \nu^T) = \text{span}\{(1, 1)^T\}$ for this mechanism.

It is more difficult to identify general classes of systems for which there exists an $\Omega > 0$, but here is one example. Suppose that $p \leq n$ and that the complexes are independent. Then (26) has a solution but generally it is not positive. However, if ν^T has the form

$$\nu^t = [A_0 \mid A_1]$$

where A_0 is $p \times p$ and $\rho(A_0) = p$, then

$$\nu^T \Omega = [A_0 \mid A_1] \begin{pmatrix} \Omega_1 \\ \Omega_2 \end{pmatrix} = A_0 \Omega_1 + A_1 \Omega_2 = u$$

and so

$$\Omega_1 = A_0^{-1}(u - A_1 \Omega_2).$$

If $\Omega_2 > 0$, then $A_1 \Omega_2 > 0$ and $u - A_1 \Omega_2$ can be made positive by choosing the ω_j 's sufficiently large. Therefore $\Omega_1 > 0$ if A_0^{-1} is non-negative. It is difficult to characterize the class of matrices that have a non-negative inverse, but it certainly contains the diagonal matrices with positive diagonal elements. This will be true of A_0 whenever the species and complexes can be ordered so that the q^{th} complex, $q = 1, \dots, p$, contains at least the q^{th} species and perhaps one or more of the $(p+1)^{\text{st}}$ through n^{th} species. Evidently this is true if all complexes are non-constant species.

5 Dynamical Equivalence of Networks.

The stoichiometric and incidence matrices associated with a network are fixed once a choice of complexes and reactions is made, but even if the reactions are all elementary, they need not be independent. This raises the more general question as to what transformations of the complexes, reactions and rate functions preserve the dynamical behavior of the network. The dynamical behavior is completely determined by the triple $(\nu, E, P(c))$, and two networks characterized by $(\nu, E, P(c))$ and $(\nu', E', P'(c))$ respectively, are said to be *dynamically equivalent* if

(i) The domains of $P(c)$ and $P'(c)$ are identical.

and

(ii) $\nu E P(c) = \nu' E' P'(c)$ for all c in the domain of P .

If one side of the equation in (ii) vanishes identically they both must, and therefore i_3 is invariant under transformations that preserve equivalence (equivalence transformations hereafter). It follows from (10) that the subspace spanned by the kinematic invariants is also unchanged, and this implies that both $i_1 + i_2$ and the reaction subspace remain fixed.

Three types of equivalence transformations are of interest here, namely

- (1) The identification of equal complexes.
- (2) The removal of cycles in the graph.
- (3) The removal of elements in $\mathcal{N}(\nu) \cap R(E)$.

The first of these leaves r fixed and changes p and perhaps q , the second changes r and leaves p and q fixed, and the third changes r and perhaps p and q . The first type is not as trivial as it may appear to be at first glance, because 'equal' complexes need only be equal with respect to the time-dependent species.

Let $\nu_{(i)}$ denote a column of ν . Let $\mathcal{E}_{(i)}$ denote a row of E , and suppose that $\nu_{(i)} = \nu_{(j)}$ for some pair $(i, j), i \neq j$. Then

$$\nu\mathcal{E} = [\nu_{(1)} \dots \nu_{(i)} \nu_{(j)} \dots \nu_{(p)}] \begin{bmatrix} \mathcal{E}_{(1)} \\ \vdots \\ \mathcal{E}_{(p)} \end{bmatrix}$$

can be contracted to

$$\nu'\mathcal{E}' = [\nu_{(1)} \dots \nu_{(i)} \dots \nu_{(p)}] \begin{bmatrix} \mathcal{E}_{(\mathcal{E})} \\ \vdots \\ \mathcal{E}_{(i)} + \mathcal{E}_{(j)} \\ \vdots \\ \mathcal{E}_{(p)} \end{bmatrix}$$

\mathcal{E}' is the incidence matrix of a graph \mathcal{G}' derived from \mathcal{G} by moving all edges incident at vertex j to vertex i and deleting vertex j . \mathcal{G}' may have cycles even if \mathcal{G} is acyclic, but because the reaction $C(i) \rightarrow C(j)$ is not admitted when $\nu_{(i)} = \nu_{(j)}$, \mathcal{G}' has no cycles of length one. Furthermore, if both i and j react to k , \mathcal{G}' will have two edges from i to k . This creates a cycle, which is removed in the next step. In any case, $\nu\mathcal{E} = \nu'\mathcal{E}'$, and since the P_i 's are unchanged, the foregoing is an equivalence transformation. By applying this identification procedure repeatedly if necessary, the number of null complexes in a network can always be reduced to one.

The removal of cycles, which are elements in $\mathcal{N}(\mathcal{E})$, proceeds as follows. Choose a spanning tree in \mathcal{G} and write

$$\mathcal{E} = [\mathcal{E}_2 \mid \mathcal{E}_1]$$

where \mathcal{E}_1 contains the edges in the chosen tree. A set of $p - q$ independent cutsets can be chosen so that every edge of the tree is in one and only one cutset, and so that the orientation of the cutset through a tree edge agrees with the orientation of the tree edge. The resulting cutsets comprise a *fundamental set* and the cutset matrix for this set is

$$\mathcal{Q} = [\mathcal{Q}_1 \mid I], \tag{32}$$

where \mathcal{Q}_1 contains the edges not in the tree. Since an edge of the tree intersects exactly one cutset, it is easy to see that $\mathcal{E}_2 = \mathcal{E}_1\mathcal{Q}_1$, and therefore

$$\mathcal{E} = \mathcal{E}_1[\mathcal{Q}_1 \mid I]. \tag{33}$$

Consequently,

$$\nu\mathcal{E}P(c) = \nu\mathcal{E}_1[\mathcal{Q}_1 \mid I]P(c) = \nu\mathcal{E}_1P'(c) \tag{34}$$

where

$$P'(c) = [\mathcal{Q}_1 \mid I]P(c). \tag{35}$$

Since \mathcal{E}_1 is the incidence matrix for a tree, the new network, whose incidence matrix is \mathcal{E}_1 , contains no cycles. However, in removing the cycles we have to reassign the rates on edges not in the tree to the tree edges. The definition

and

$$\nu = \begin{bmatrix} 1 & 0 & 2 & 3 & 0 \\ 0 & 1 & 1 & 0 & 0 \end{bmatrix}$$

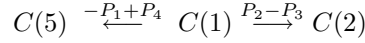
It follows that $\rho(\nu\mathcal{E}) = 2$, which implies that there are no kinematic invariants, as could be predicted from Proposition 3. Since $\rho(\mathcal{E}) = p - 1 = 3$, $\delta = 1$ and one finds that

$$N(\nu) \cap R(\mathcal{E}) = \text{span}\{(-1, 1 - 1, 1, 0)^T\}.$$

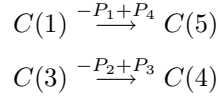
If we choose to retain reactions 2 and 4, then

$$D = \begin{bmatrix} 1 & 0 & 1 \\ 0 & 0 & 1 \\ 0 & 1 & 0 \end{bmatrix}$$

and some elementary computations show that \mathcal{G}' is given by



where the rates are as indicated. If we retain reactions 3 and 4 then \mathcal{G}' is



The reader can analyze the remaining possibility.

The foregoing example shows that the number of complexes and the number of components may be different in two equivalent networks, each of which has $\delta' = 0$, distinct complexes, and an acyclic graph. Of course these numbers are not independent, because s is invariant under equivalence transformations and so when $\delta' = 0$, $q' = p' - s$. However, r' is the same for equivalent networks if both are acyclic.

We noted earlier that $i_1 + i_2$ is invariant under equivalence transformations, but in fact both i_1 and i_2 are separately invariant. Identifying equal complexes changes the number of rows of ν^T but only removes redundant equations in the system $\nu^T\Omega = 0$, and therefore does not alter $\dim \mathcal{N}(\nu^T)$. Thus i_1 remains fixed and so also does i_2 .

6 Necessary Conditions for the Existence of a Steady State.

6.1 The Relationship Between Local and Global Deficiency.

When there is no positive Ω in $\mathcal{N}(\mathcal{E}^T\nu^T)$, as happens for instance when there are null complexes in the network, arguments for the existence of a steady state based on the compactness of the reaction simplex are not applicable, and it is much harder to answer the existence question affirmatively. Indeed, it is easier to give sufficient conditions for the absence of any steady state, and such conditions are derived in this section. From an analytical standpoint it is easier to treat the case in which the P_i 's are nonnegative, and therefore we assume at the outset that any network transformations that are made preserve this nonnegativity.

It can be seen from (4) that there are three distinct classes of steady states c^s of the network, defined by the sets

$$\begin{aligned} \mathcal{S}_0 &\equiv \{c^s \in \overline{R_n^+} \mid P(c^s) = 0\} \\ \mathcal{S}_1 &\equiv \{c^s \in \overline{R_n^+} \mid \mathcal{E}P(c^s) = 0, P(c^s) \geq 0\} \\ \mathcal{S}_2 &\equiv \{c^s \in \overline{R_n^+} \mid \nu\mathcal{E}P(c^s) = 0, \mathcal{E}P(c^s) \neq 0\}. \end{aligned} \tag{37}$$

The first of these is empty when there are non-vanishing constant inputs to the network. Furthermore, since the forward and reverse reactions of a reversible pair are treated separately, a steady state for such a reaction pair would fall into this class only if the rate of each reaction vanished separately. This would be unusual but it can happen in autocatalytic reactions or in reactions involving threshold phenomena.

The net rate of formation of each complex, as formally defined by (3), vanishes at a steady state in the second class. In circuit-theoretic terms, the flux of $C(i)$ into the i^{th} vertex balances the flux away from the i^{th} vertex and Kirchoff's current law applies (Oster and Perelson 1974). In the terminology used by Horn and Jackson (1972), the system is *complex balanced* at steady states in \mathcal{S}_1 . At any $c^s \in \mathcal{S}_2$, the rate of formation of each species vanishes but there exists at least one vertex in the graph at which the net flux of the complex is nonzero.

Suppose, as in Section 3.2, that \mathcal{G} has q components, and let P_α be the number of vertices in \mathcal{G}_α . Order the complexes in accordance with the partition of \mathcal{G} into components and partition ν, \mathcal{E} and P to conform with this ordering; then (4) can be written

$$\frac{dc}{dt} = [\nu^1 \mid \nu^2 \mid \dots \mid \nu^q] \begin{bmatrix} \mathcal{E}^1 & & 0 \\ & \mathcal{E}^2 & \\ 0 & & \mathcal{E}^q \end{bmatrix} = \sum_{\alpha} \nu^{\alpha} \mathcal{E}^{\alpha} P^{\alpha}. \quad (38)$$

Here ν^{α} is $n \times p$, \mathcal{E}^{α} is $p_{\alpha} \times r_{\alpha}$, and P^{α} is $r_{\alpha} \times 1$, where r_{α} is the number of reactions in \mathcal{G}_{α} . Each \mathcal{E}^{α} is the incidence matrix for the corresponding subgraph \mathcal{G}_{α} and $\rho(\mathcal{E}^{\alpha}) = p_{\alpha} - 1$. As before, the partition leads to a direct sum decomposition of C_0 and C_1 .

The columns of each $\nu^{\alpha} \mathcal{E}^{\alpha}$ are the stoichiometric vectors for the reactions in \mathcal{G}_{α} , and the number of these that are independent, call it s_{α} , is given by $\rho(\nu^{\alpha} \mathcal{E}^{\alpha})$. Since $\rho(\mathcal{E}^{\alpha}) = p_{\alpha} - 1$, Sylvester's law (Minc and Marcus 1964) implies that s_{α} cannot exceed $p_{\alpha} - 1$. The *local deficiency* δ_{α} is defined as

$$\begin{aligned} \delta_{\alpha} &= p_{\alpha} - 1 - s_{\alpha} \\ &= \dim \mathcal{R}(\mathcal{E}^{\alpha}) - \dim \mathcal{R}(\nu^{\alpha} \mathcal{E}^{\alpha}) \\ &= \dim[\mathcal{N}(\nu^{\alpha}) \cap \mathcal{R}(\mathcal{E}^{\alpha})] \end{aligned}$$

and it vanishes if and only if the number of independent reactions in \mathcal{G}_{α} is exactly the number set by the graph structure, namely, $p_{\alpha} - 1$. The total number of independent reactions computed for all components is $\rho(\nu \mathcal{E})$ and it follows from (36) that

$$s = \rho(\nu \mathcal{E}) \leq \sum_{\alpha} s_{\alpha} \leq p - q.$$

Therefore the global deficiency δ is

$$\delta = p - q - s \geq p - q - \sum_{\alpha} s_{\alpha} = \sum_{\alpha} \delta_{\alpha}$$

and since $\delta_{\alpha} \geq 0$, $\delta = 0$ implies that $\delta_{\alpha} = 0$, $\alpha = 1, \dots, q$, **but not conversely**. For future reference, we summarize these facts and others in the following proposition.

Proposition 6 Let \mathcal{G} be the graph of a reaction network with p complexes, r reactions and q components. Then

- (i) $r_{\alpha} \geq p_{\alpha} - 1 \geq s_{\alpha}$
 $r \geq p - q \geq s$
- (ii) $\delta_{\alpha} = p_{\alpha} - 1 - s_{\alpha} \geq 0$
 $\delta = p - q - s \geq \sum \delta_{\alpha} \geq 0$
- (iii) $s = \sum s_{\alpha}$ if and only if $\delta = \sum \delta_{\alpha}$.

(iv) $\delta_\alpha = 0$ iff $\mathcal{N}(\nu^\alpha) \cap \mathcal{R}(\mathcal{E}^\alpha) = \{0\}$

$\delta = 0$ iff $\mathcal{N}(\nu) \cap \mathcal{R}(\mathcal{E}) = \{0\}$

(v) $\delta = 0$ implies $\delta_\alpha = 0, \alpha = 1, \dots, q$.

It follows from (i) that the number of dependent reactions $r_\alpha - s_\alpha$ in component α is at least as large as the number of oriented cycles in \mathcal{G}_α , and likewise for the entire graph. The excess number δ_α certainly vanishes if the complexes in that component are independent, but this is not a necessary condition. The strongest conclusions that can be reached are as follows.

Proposition 7 Let \mathcal{G} be as in Proposition 5. Then

(i) if there is exactly one null complex in \mathcal{G}_α and if $\dim \mathcal{N}(\nu^\alpha) = 1$, then $\delta_\alpha = 0$

(ii) if $\delta_\alpha = 0$ then $\dim \mathcal{N}(\nu^\alpha) \leq 1$

(iii) if $\delta = 0$ then $\dim \mathcal{N}(\nu) \leq q$

(iv) If $\delta_\alpha = 0, \alpha = 1, \dots, q$, and if the set of non-null complexes for the entire network is linearly independent, then $\delta = 0$.

Proof: Let $K^\alpha \equiv \overline{C_{0\alpha}^+} \cup \{-\overline{C_{0\alpha}^+}\}$. Since $\sum_i y_i = 0$ for any $y \in \mathcal{R}(\mathcal{E}^\alpha)$,

$$\mathcal{R}(\mathcal{E}^\alpha) \cap K^\alpha = \{0\}$$

and therefore $\delta_\alpha = 0$ if all $x \in \mathcal{N}(\nu^\alpha)$ lie in K^α . If $\dim \mathcal{N}(\nu^\alpha) = 1$ and there is exactly one null complex in \mathcal{G}_α , which we label as the p_α^{th} , then $(0, 0, \dots, 0, 1)^T \in \mathcal{N}(\nu^\alpha)$ and therefore $\delta_\alpha = 0$.

If $\delta_\alpha = 0$ then $\mathcal{R}(\mathcal{E}^\alpha) \cap \mathcal{N}(\nu^\alpha) = \{0\}$, and since $\mathcal{N}((\mathcal{E}^\alpha)^T) \cap \mathcal{N}(\nu^\alpha) = \{0\}$, it follows that $\dim \mathcal{N}(\nu^\alpha) \leq \min\{1, p_\alpha - 1\}$. Since there are no non-reacting complexes, $p_\alpha \geq 2$ and consequently,

$$\dim \mathcal{N}(\nu^\alpha) \leq 1.$$

If $\delta = 0$, a modification of the foregoing shows that

$$\dim \mathcal{N}(\nu) \leq \min\{q, p - q\}$$

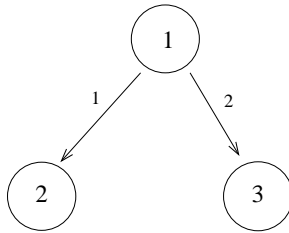
and since $p_\alpha \geq 2, p \geq 2q$. Therefore

$$\dim \mathcal{N}(\nu) \leq q.$$

Under the conditions in (iv),

$$\rho(\nu\mathcal{E}) = \sum_\alpha \rho(\nu^\alpha \mathcal{E}^\alpha) = \sum \rho(\mathcal{E}^\alpha) = \rho(\mathcal{E}).$$

It follows from (ii) that $\delta_\alpha > 0$ if there is more than one dependent complex in any component. However, we know from Section 4 that whenever the dependencies are due to the presence of several null complexes, the network is dynamically equivalent to one with only one null complex. If $\dim \mathcal{N}(\nu^\alpha) = 1$ for every component in the new graph, the according to (i), $\delta_\alpha = 0$ for every component. The conclusions in (i) and (ii) are the best possible in that $\dim \mathcal{N}(\nu^\alpha) = 1$ does not by itself imply that $\delta_\alpha = 0$. This is illustrated in the following example. Suppose that $(n, p, q, r) = (2, 3, 1, 2)$ and that \mathcal{G}, ν , and \mathcal{E} are as follows.



$$\nu = \begin{bmatrix} \nu_{11} & \nu_{12} & \nu_{13} \\ \nu_{21} & \nu_{22} & \nu_{23} \end{bmatrix} \quad \mathcal{E} = \begin{bmatrix} -1 & -1 \\ 1 & 0 \\ 0 & 1 \end{bmatrix}$$

Suppose that $\rho(\nu) = 2$, and that

$$\nu_{(3)} = \alpha\nu_{(1)} + \beta\nu_{(2)}.$$

One finds that $\det \nu\mathcal{E} = (1 - (\alpha + \beta))[\nu_{11}\nu_{22} - \nu_{12}\nu_{21}]$, and consequently

$$\delta = \begin{cases} 0 & \text{if } \alpha + \beta \neq 1 \\ 1 & \text{if } \alpha + \beta = 1. \end{cases}$$

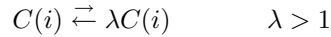
If α and β are non-zero and $\alpha + \beta \neq 1$, the example also illustrates the fact that linear independence of the non-null complexes is not a necessary condition for having $\delta = 0$. However, according to (ii) and (iii) of Proposition 7, there can be at most one linear relation between the stoichiometric vectors of the complexes in component α if δ_α is to be zero, and at most q overall if δ is to be zero. Because $\sum y_i = 0$ for any $y \in \mathcal{N}(\mathcal{E}^\alpha)$, the most general linear relationship between the complexes that is compatible with $\delta_\alpha = 0$ is of the form

$$\sum_{i=1}^p y_i^\alpha \nu_{(i)}^\alpha = 0, \quad \sum_{i=1}^p y_i^\alpha \neq 0. \quad (39)$$

If these are satisfied for all α and there are no inter-component relations of the form

$$\sum_{i=1}^p y_i \nu_{(i)} =, \quad \sum_{i=1}^p y_i = 0, \quad (40)$$

then $\delta = 0$ as well. Thus an autocatalytic reaction of the form



does not, by itself, lead to a non-zero deficiency. However, the Wegscheider mechanism discussed in Section 3 has two inter-component relations between the complexes and therefore $\delta > 0$ for this mechanism, even though $\delta_\alpha = 0$ for each component.

6.2 Nonexistence of Balanced Flows

At a steady state $c^s \in \mathcal{S}_2$, the flow $P(c^s)$ satisfies

$$\mathcal{E}P(c^s) = 0, \quad P(c^s) \geq 0,$$

and the partitioned form of the equations at (38) shows that

$$\mathcal{E}^\alpha P^\alpha(c^s) = 0 \quad \alpha = 1, \dots, q. \quad (41)$$

Consequently, the flow $P^\alpha(c^s)$ on each component of \mathcal{G} is a balanced nonnegative flow and is strictly nonnegative on at least one component. Our next objective is to determine when such flows cannot exist, and for this purpose we require the following special case of a theorem due to (Motzkin 1936).¹¹

Theorem 8 Let A be a non-zero matrix. Then either $Ax = 0, x \geq 0$ has a solution or $A^T y > 0$ has a solution, but never both.

¹¹The complete statement of this theorem, Stiemke's theorem, and more general alternative theorems can be found in (Mangasarian 1969).

Theorem 9 Let \mathcal{G} be the graph of a reaction network with p complexes, r reactions and q components.

- (i) If \mathcal{G} is acyclic then there exists no strictly nonnegative balanced flow.
- (ii) There exists a positive balanced flow if and only if every component \mathcal{G}_α of \mathcal{G} is strongly connected.

Proof. : To prove (i), all we have to do is show that

$$\mathcal{E}^T y > 0 \quad (42)$$

has a solution when \mathcal{G} is acyclic. Without loss of generality, we can assume that $\alpha = 1$, for otherwise we apply the argument to each component. In order that there be a solution, it is necessary and sufficient that y satisfy

$$-y_i + y_j > 0 \quad (43)$$

for every ordered pair (i, j) in the edge set of \mathcal{G} . To construct a y that satisfies (43), we proceed as follows. Since \mathcal{G} is acyclic, there is at least one vertex V_j with $d_j^+ = 0$ (an 'initial' vertex) and at least one V_k with $d_k^+ = 0$ (a 'terminal' vertex). Choose a directed path from an initial vertex V_1 to a terminal vertex V_k and, beginning with $y_1 = 1$, define $y_{k+1} = y_k + 1$ for those vertices on the chosen path. If there is only one such path through \mathcal{G} this provides the desired y . If more than one path leaves V_1 , or if there is more than one initial vertex, repeat the procedure for each path. If more than one path is incident at some vertex V_j , assign y_j the largest of the values associated with the paths incident at V_j and repeat the foregoing procedure for each path leaving V_j . The resulting y satisfies (40) and this proves (i).

Since \mathcal{G}_α is strongly connected if and only if there exists a closed directed edge sequence that contains all the edges, it follows that a positive balanced flow exists when every \mathcal{G}_α is strongly connected. To prove the converse, we show that if some \mathcal{G}_α is not strongly connected then there exists no positive balanced flow. This will follow from Stiemke's theorem if we can show that

$$\mathcal{E}^T y \geq 0 \quad (44)$$

has a solution, for then

$$\mathcal{E}z = 0, \quad z > 0$$

has none. We can again assume that $\alpha = 1$ without loss of generality. Partition \mathcal{G}_α into strong component $\mathcal{G}_{\alpha\beta}$ as in 3.2, and then (44) becomes

$$\mathcal{E}^T y = \begin{pmatrix} \mathcal{E}_{11}^T & 0 & \mathcal{E}_{31}^T & \mathcal{E}_{41}^T & \mathcal{E}_{51}^T & \mathcal{E}_{61}^T \\ 0 & \mathcal{E}_{22}^T & \mathcal{E}_{32}^T & \mathcal{E}_{42}^T & \mathcal{E}_{52}^T & \mathcal{E}_{62}^T \\ 0 & 0 & \mathcal{E}_{33}^T & \mathcal{E}_{43}^T & \mathcal{E}_{53}^T & \mathcal{E}_{63}^T \\ 0 & 0 & 0 & \mathcal{E}_{44}^T & \mathcal{E}_{54}^T & \mathcal{E}_{64}^T \\ 0 & 0 & 0 & 0 & \mathcal{E}_{55}^T & 0 \\ 0 & 0 & 0 & 0 & 0 & \mathcal{E}_{66}^T \end{pmatrix} \begin{pmatrix} y_1 \\ y_2 \\ y_3 \\ y_4 \\ y_5 \\ y_6 \end{pmatrix} \geq 0.$$

(As before, we illustrate the case in which there are two each of sources, sinks and internal strong components, but the arguments used apply in general.) Now recall that \mathcal{E}_{55} and \mathcal{E}_{66} are the incidence matrices for strong components, and therefore

$$\mathcal{E}_{jj}^T y_j \geq 0 \quad j = 5, 6$$

has no solution because

$$\mathcal{E}_{jj} z_j = 0, \quad z_j > 0$$

has a solution by virtue of the 'if' part of (ii) applied to the subgraphs. Furthermore, recall that the off-diagonal blocks are non-negative, and since \mathcal{G}_α is not strongly connected and $p > 1$, there is at least one such block that is not identically zero. Therefore, if we choose

$$\begin{aligned} y_j &= 0 \quad j = 1, \dots, 4 \\ y_j &= u_j \quad j = 5, 6 \end{aligned}$$

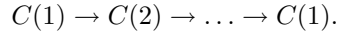
then

$$\mathcal{E}_{jj}^T y_j = 0 \quad j = 1, \dots, 6$$

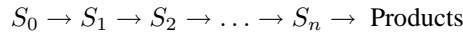
and for at least one pair (i, j)

$$\mathcal{E}_{ij}^T y_j \geq 0.$$

The theorem implies that \mathcal{S}_1 can be nonempty only if at least one component of \mathcal{G} contain a cyclic sequence of reactions of the form



'Apparent' cycles may in fact not be cyclic in the foregoing sense. For instance, the feedback system



in which all reactions shown are irreversible, is acyclic when the rate function for the first reaction is taken to be $f(S_n) \cdot S_0$ (Tyson and Othmer 1978). However if the concentrations of S_0 and the products are time invariant and if the associated complexes are identified, the resulting network is strongly connected.

6.3 Conditions under which $\mathcal{S}_2 = \phi$.

The flows corresponding to steady state in \mathcal{S}_2 contains both cycles and cutsets and therefore the steady-state version of (38) reads

$$0 = \sum_{\alpha} \nu^{\alpha} \mathcal{E}^{\alpha} P^{\alpha}(c^s) = \sum_{\alpha} \nu^{\alpha} \mathcal{E}^{\alpha} [(\mathcal{B}^{\alpha})^T w^{\alpha} + (\mathcal{Q}^{\alpha})^T z^{\alpha}] = \sum_{\alpha} \nu^{\alpha} \mathcal{E}^{\alpha} (\mathcal{Q}^{\alpha})^T z^{\alpha} \quad \alpha = 1, \dots, q \quad (45)$$

wherein $z^{\alpha} \neq 0$ for at least one α . The sum vanishes either if every term vanishes or if there are at least two nonzero terms whose sum vanishes. In the former case the steady state flow is said to be *locally-compensated* because the unbalanced part of the flow is annihilated by $\nu^{\alpha} \mathcal{E}^{\alpha}$ on every component \mathcal{G}_{α} . The flow is only *globally-compensated* at c^s in the latter case because there is at least one species in the system for which the net rate of production in one component of \mathcal{G} is compensated for by a net rate of consumption in another component. In either case the flow can be balanced on as many as m components, where $m = q - 1$ ($q - 2$) for a locally-compensated (globally-compensated) steady state flow. Of course all flows that are balanced are automatically locally-compensated, and because $\mathcal{R}(\mathcal{E}) = \oplus \mathcal{R}(\mathcal{E}^{\alpha})$, balanced flows are always balanced component-wise (in fact vertex-wise).

If $\delta_{\alpha}^{\alpha} = 0$ for all α there are certainly no locally-compensated flows that are not balanced. This occurs, for instance when $\mathcal{N}(\nu^{\alpha}) = \{0\}$ for all α , in which case the complexes within each component are independent. In order for δ to be positive, it is necessary that $(\nu \mathcal{E}) \subset \oplus \mathcal{R}(\nu^{\alpha} \mathcal{E}^{\alpha})$, i.e., the reaction subspace for the entire network must be a proper subspace of the direct sum of those for the components. Furthermore,

$$\mathcal{N}(\nu) = \{y \in C_0 \mid y = (y^1, \dots, y^q)^T, \nu y = 0, \nu^{\alpha} y^{\alpha} \neq 0 \text{ for } y^{\alpha} \neq 0\} \equiv \mathcal{N}_0 \quad (46)$$

in this case. Examples of this case are given by the Prigogine-Lefever mechanism and by the Wegscheider mechanism, a special case of which will be discussed later.

In general

$$\mathcal{N}(\nu) = \{\oplus_{\alpha} \mathcal{N}(\nu^{\alpha})\} \oplus \mathcal{N}_0 \quad (47)$$

and the opposite extreme occurs when $\mathcal{N}_0 = \{0\}$. Now there are no inter-component linear combinations of complexes that are zero, and all steady-state flows that are unbalanced are necessarily locally-compensated. In order for δ to be positive, there must be a $\delta_{\alpha} \neq 0$ for at least one α , and here $\mathcal{R}(\nu\mathcal{E}) = \oplus \mathcal{R}(\nu^{\alpha}\mathcal{E}^{\alpha})$. Thus $s = \sum s_{\alpha}$ and by Proposition 5, $\delta = \sum \delta_{\alpha}$.

The foregoing provides sufficient conditions for the absence of locally-compensated flows ($\delta_{\alpha} = 0$ for all α), sufficient conditions under which there are no globally-compensated flows ($\delta = \sum \delta_{\alpha}$), and sufficient conditions for $S_2 = \phi$ ($\delta = 0$). However, these conditions are not necessary, as a later example will illustrate. A sharper set of conditions is obtained as follows. Let

$$K_1^{\alpha} \equiv \{y^{\alpha} \in C_{0\alpha} \mid y^{\alpha} = \mathcal{E}^{\alpha}x^{\alpha}, x^{\alpha} \in C_{1\alpha}, x^{\alpha} \geq 0\}. \quad (48)$$

Then the equations

$$\nu^{\alpha}\mathcal{E}^{\alpha}x^{\alpha} = 0 \quad \alpha = 1, \dots, q \quad (49)$$

have a solution for which $\mathcal{E}^{\alpha}x^{\alpha} \neq 0$ for at least one α only if there is an α for which

$$\mathcal{N}(\nu^{\alpha}) \cap \{K_1^{\alpha} - \{0\}\} \neq \phi. \quad (50)$$

Therefore there is no locally-compensated flow that is not balanced on at least one component \mathcal{G}_{α} if and only if

$$\mathcal{N}(\nu^{\alpha}) \cap K_1^{\alpha} = \{0\}, \quad \alpha = 1, \dots, q. \quad (51)$$

Similarly, $S_2 = \phi$ if

$$\mathcal{N}(\nu) \cap K_1 = \{0\}, \quad (52)$$

where

$$K_1 \equiv \{y \in C_0 \mid y = \mathcal{E}x, x \in C_1, x \geq 0\}. \quad (53)$$

Certainly $\delta_{\alpha} = 0 \alpha = 1, \dots, q$ implies (51) and $\delta = 0$ implies (49), but not conversely, as the following example illustrates.

Consider again the example following Proposition 6, and suppose that $\alpha + \beta = 1$, which means that $\delta = 1$. The cone K_1 is generated by the vectors $(-1, 1, 0)^T$ and $(-1, 0, 1)^T$ and $\mathcal{N}(\nu)$ is spanned by $(\alpha, 1 - \alpha, -1)^T$. Some elementary computation shows that when $0 \leq \alpha \leq 1$ there is no pair (λ_1, λ_2) with $\text{sgn } \lambda_1 = \text{sgn } \lambda_2$ such that

$$\lambda_1 \begin{pmatrix} -1 \\ 1 \\ 0 \end{pmatrix} + \lambda_2 \begin{pmatrix} -1 \\ 0 \\ 1 \end{pmatrix} = \begin{pmatrix} \alpha \\ 1 - \alpha \\ -1 \end{pmatrix}$$

Therefore, for this choice of (α, β)

$$\mathcal{N}(\nu) \cap \{K_1 - \{0\}\} = \phi$$

and so $S_2 = \phi$. Moreover, since \mathcal{G} is acyclic, either there is a $c^s \in \overline{R_2^+}$ at which the flow on both edges vanishes or there is no steady state.

When all reactions in every component are reversible, the negative of any column of \mathcal{E} is also a column of \mathcal{E} , and so

$$\begin{aligned} \mathcal{E}^{\alpha}x &= [\mathcal{E}_{(1)} \quad -\mathcal{E}_{(1)} \quad \mathcal{E}_{(2)} \quad -\mathcal{E}_{(2)} \quad \dots] \begin{bmatrix} x_1 \\ x_2 \\ \vdots \\ x_r \end{bmatrix} \\ &= (x_1 - x_2)\mathcal{E}_{(1)} + (x_3 - x_4)\mathcal{E}_{(2)} + \dots \end{aligned}$$

Consequently, it cannot happen that

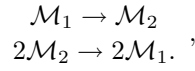
$$\mathcal{N}(\nu^\alpha) \cap \{K_1^\alpha\} = \{0\} \quad (54)$$

even though $\delta_\alpha > 0$. Of course this only asserts the existence of a vector $x \geq 0$ such that $\mathcal{E}^\alpha x \neq 0$ and $\nu^\alpha \mathcal{E}^\alpha x = 0$; it does not imply that for a given choice of rate functions a steady state c^s exists at which the flow is unbalanced. Another case in which (51) does not occur for $\delta_\alpha > 0$ is when \mathcal{G}_α is strongly-connected. Then Theorem 2 shows that there is an $x_0 > 0$ such that $\mathcal{E}^\alpha x_0 = 0$, and given any $x_1 \in \mathcal{N}(\nu^\alpha) \cap \mathcal{R}(\mathcal{E}^\alpha)$, let $x = x_1 + \lambda x_0$ with $\lambda > 0$ and sufficiently large. Thus x is a locally-compensated, unbalanced, non-negative flow, but again, this is not an existence result for any given choice of rate functions.

Earlier we interpreted δ_α as the excess of the actual number of dependent reactions in \mathcal{G}_α over the number set by the structure of \mathcal{G}_α . An alternate interpretation is as follows. Since $\dim \mathcal{N}(\nu^\alpha \mathcal{E}^\alpha) = r_\alpha - s_\alpha$, there exist $r_\alpha - s_\alpha$ linearly independent flows that are annihilated by $\nu^\alpha \mathcal{E}^\alpha$. Since every oriented cycle in \mathcal{G}_α lies in $\mathcal{N}(\nu^\alpha \mathcal{E}^\alpha)$ there are

$$r_\alpha - s_\alpha - (r_\alpha - p_\alpha + 1) = p_\alpha - s_\alpha - 1 = \delta_\alpha$$

independent flows in $\mathcal{N}(\nu^\alpha \mathcal{E}^\alpha)$ that are not balanced. These are necessarily cutsets, in view of the decomposition of $C_{1\alpha}$, and thus δ_α represents the maximum number of independent cutsets that can appear in any flow that is annihilated by $\nu^\alpha \mathcal{E}^\alpha$. Of course a particular flow need not contain that many; it could, for instance, be balanced even if $\delta_\alpha > 0$. If \mathcal{G}_α is a tree then every flow on \mathcal{G}_α is a linear combination of cutsets and if $\delta_\alpha = 0$ there is no such combination that is annihilated by $\nu^\alpha \mathcal{E}^\alpha$. In this case $\mathcal{S}_1 = \phi$ and there are no locally-compensated steady flows for $c^s \in S_2$. However S_2 need not be empty, since there may be a globally-compensated flow. As an example of this, consider the following special case of Wegscheider's mechanism:



Let $C(1) = \mathcal{M}_1, C(2) = \mathcal{M}_2, C(3) = 2\mathcal{M}_1, C(4) = 2\mathcal{M}_2$. Then the graph is



and the stoichiometric and incidence matrices are

$$\nu = \begin{bmatrix} 1 & 0 & 2 & 0 \\ 0 & 1 & 0 & 2 \end{bmatrix} \quad \mathcal{E} = \begin{bmatrix} -1 & 0 \\ 1 & 0 \\ 0 & 1 \\ 0 & -1 \end{bmatrix}$$

It is evident that $\rho(\nu^1 \mathcal{E}^1) = \rho(\mathcal{E}^1) = 1$ and that $\rho(\nu^2 \mathcal{E}^2) = \rho(\mathcal{E}^2) = 1$; thus the flow cannot be locally-compensated, nor can it be balanced unless it vanishes on all edges, since \mathcal{G} is acyclic. One finds that $\rho(\nu \mathcal{E}) = 1$, so $\delta = 1$ and S_2 need not be empty. A simple computation shows that any $z_0 \in \mathcal{N}(\nu)$ and $z_1 \in \mathcal{R}(\mathcal{E})$ must have the form

$$z_0 = \begin{bmatrix} -2u \\ -2v \\ u \\ v \end{bmatrix} \quad z_1 = \begin{bmatrix} -x \\ x \\ y \\ -y \end{bmatrix}$$

where u, v, x, y are arbitrary. Consequently

$$\mathcal{E}P(c^s) = \begin{bmatrix} -P_1(c^s) \\ P_1(c^s) \\ P_2(c^s) \\ -P_2(c^s) \end{bmatrix} \in \mathcal{N}(\nu)$$

if and only if the system

$$\begin{aligned} P_1(c^s) &= \lambda \\ P_2(c^s) &= 2\lambda \end{aligned}$$

has a solution $c^s \in \overline{R_2^+}$ for some $\lambda \geq 0$. When a solution exists $S_2 \neq \phi$; whether or not it does depends on the choice of constitutive relations.

6.4 A Flow Chart For Determining Whether Any Steady State Can Exist.

The foregoing leads to a procedure for systematically deciding whether certain types of flows can exist or whether they can be ruled out on the basis of stoichiometry and network structure alone. A schematic flow chart for such a procedure is shown in Figure 2. The conclusion that there are no positive balanced flows unless every \mathcal{G}_α is strongly connected is essentially due to Horn (1972) and is part of the zero deficiency theorem (Feinberg 1977; Feinberg 1980). That $\delta = 0$ implies $S_2 = \Phi$ also follows from Feinberg (1972), but the notions of locally- and globally-compensated flows are not used there. Neither of the foregoing authors allows rate functions that vanish at positive concentrations and the set S_0 does not arise in their analyses.

The problem of constructing and implementing the algorithms required for the tests in the flow chart will be dealt with elsewhere, but some general remarks are in order. Algorithms for deciding whether a graph is strongly-connected or acyclic exist, and the tests on the deficiency only require a comparison of the ranks of two matrices. If the complexes are independent these ranks are obviously equal and $S_2 = \phi$. A less trivial case occurs when every non-null complex is a species, for then $\mathcal{N}(\nu) = \oplus \mathcal{N}(\nu^\alpha)$ and the rank condition can be checked separately for each component \mathcal{G}_α .

The general problem of deciding when (48) and/or (49) are satisfied can be cast as the quadratic programming problem

$$\begin{aligned} \min \quad & \langle \nu x, \nu x \rangle, \\ & x \in \text{Int } K_1 \end{aligned}$$

but this aspect will not be pursued here. If all the P_i are bounded above, as for instance when the reactions are all enzyme-catalyzed and Michaelis-Menten kinetics are applicable, the minimization is over a convex polyhedron K_1^* instead of over the cone K_1

7 Discussion

When there is only one independent reaction in the system, the dynamical behavior is completely described by the solution of the scalar equation $\xi = f(\xi)$ in the extent of reaction, and a qualitative analysis is very easy. One can readily determine the steady states, the transient behavior for all initial conditions, and the sensitivity of solutions with respect to parametric changes and changes in the form of f . It is evidently important to be able to obtain similar information about general networks, for such information would enable one to predict, for instance, how the dynamical behavior changes when a catalyst in some pathway is poisoned or a new pathway is added. If good techniques for obtaining this information for large systems were available, a composite performance index that measures stability, sensitivity and efficiency could be devised, and alternative synthetic pathways could be compared. Such comparisons might well provide insights into why existing biochemical pathways and networks built from them have evolved to their present form. Unfortunately such techniques are not available yet, but it is clear that the ability to systematically analyze how the reaction phenomenology and network structure are reflected in the dynamical equations is a step toward their development. The graph-theoretic methods we introduce here seem to serve this purpose well.

Substantial progress has been made by others toward understanding special classes of reaction networks. An early attempt to obtain global results for open systems with ideal mass action kinetics was made by Shear (1967). However, as Higgins (1968) pointed out, Shear's H-function, which is actually the thermodynamic availability of the

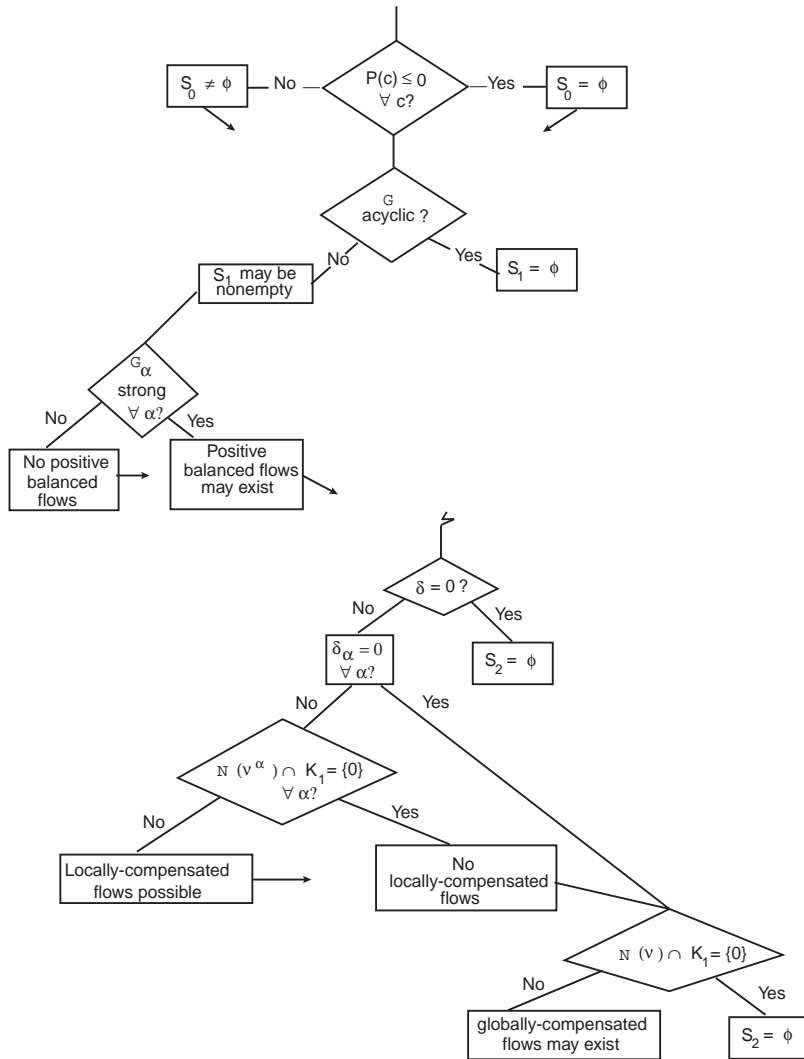


Figure 2: A flow chart for determining the existence of steady states.

system relative to an assumed steady state, is not a Lyapunov function for all systems, as Shear had claimed. In a landmark paper, Horn and Jackson (1972) showed that if a strictly positive steady state exists, the availability is a local Lyapunov function whenever the steady state flow is balanced. If one can also establish that there are no equilibria on the boundary of the simplex, and if there is an equilibrium point in the interior of the simplex, then it is unique and globally stable. Our formulation was originally motivated by a desire to extend these results to networks with mass action kinetics in general, non-ideal solutions, and to identify other classes of networks for which similar conclusions can be reached. The latter problem is particularly important in the biological context, where the rate functions in a reduced description of a network are often rational functions, and feedback control within and between pathways is the rule rather than the exception. We have made some progress on the analysis of more general networks and in sequels to this paper we will show

- (i) that the availability is a local Lyapunov function for a more general class of kinetics than ideal mass-action type, provided the flow is balanced at the steady state

- (ii) that certain networks with monotone rate functions have a unique steady state, and when they are forced periodically, they have a unique periodic solution whose period is that of the driving frequency (thus sub- and super-harmonic solutions are ruled out.)
- (iii) how the cycle structure of the Jacobian of $\nu EP(c)$ is related to the structure of the graph and the feedback present, and how these affect the stability of a steady state according to a linear analysis.

A by-product of our approach is that many of the results due to Horn and Jackson (1972), Horn (1972) and Feinberg (1977, 1980) can be obtained more directly. The separation of the usual stoichiometric matrix into the factors ν and E makes it clear why the case $\delta = 0$ is relatively easy to treat, and new interpretations of the deficiency emerge. The general representation of a balanced flow leads to an easy proof that the availability is a Lyapunov function under certain conditions, and Theorem 2 can be used to give an easy proof of parts of the zero-deficiency theorem. This is not to detract from previous work, but only to illustrate that the results become more transparent when the network structure and reaction phenomenology are separated more clearly.

Ours is not the first application of graph-theoretic techniques to the analysis of reaction networks, and in conclusion we shall mention some related work. Aris (1965) developed the algebraic approach to the analysis of reacting systems and Sellers (1966) has applied ideas from algebraic topology and graph theory to the problem. The network thermodynamics approach developed in Oster and Perelson (1974) and Perelson and Oster (1974), in which bond graphs are used to represent reactions, is related to Sellers' work. The underlying graph structure is quite different from ours, in that species rather than complexes are assigned to nodes, and the representation of chemical reactions is more complicated. Horn (1973a) recognized that networks could be represented by graphs in which the nodes represent complexes, but he did not exploit this fact in analyzing the structure of the dynamical equations. He has also used species graphs (those in which the nodes represent species) to analyze systems in which all complexes have at most two species (Horn 1973b). Williamowski and Rössler (1978) and Williamowski (1978) have continued the latter line of investigation and have enumerated the different types of second-order, ideal, mass-action kinetic networks. Toth (1979) has obtained results on the types of systems that lead to gradient flows. (1978) and (1973) make extensive use of network structure in analyzing the case in which the rate functions are represented by Boolean functions. This approach is useful in cases where the rate laws model switches, but in many cases this is too crude an approximation to reality.

The linear problem that arises either when the kinetics are first-order or when the stability of a steady state is investigated has been treated by several authors. Hill (1970) has used results from the topological analysis of linear systems to obtain representations of the steady state concentrations and fluxes for any system of reversible first-order reactions in terms of weights on directed trees in the graph. A similar approach can be used to represent the rate functions $R(c^s)$ for any mass-action-like kinetics, provided that the flow is balanced. Beretta et. al. (1979) used a technique due to Hyver (1980) to identify a class of mass action kinetics for which a steady state, if one exists, is always stable according to linear theory. Their results complement those of Horn and Jackson in that it is not necessary to assume that the deficiency is zero or that the flow is balanced. Clarke (1980) provides a comprehensive review of his own and related work on the stability problem. He also observes that the usual stoichiometric matrix can be written in factored form, as we do, but does not make use of this fact. The relation of his work on the linear problem to our approach will be discussed in a future paper devoted to the linear problem.

ACKNOWLEDGEMENTS

This paper is an outgrowth of a course offered at Rutgers University in early 1979. I am indebted to the participants, N. Greenbaun, Z. Kádas and P. Monk, for their questions and comments, which resulted in various extensions and clarifications. This work was partially supported under Grants GM21558 and GM 29123 from the National Institutes of Health.

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