GRAPH THEORETIC APPROACHES TO INJECTIVITY IN CHEMICAL REACTION SYSTEMS

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Abstract. In this paper we discuss the question of how to decide when a system of chemical reactions is incapable of admitting multiple equilibria. Our results relate previously described linear algebraic and graph theoretic conditions for injectivity of chemical reaction systems. After developing a translation between the two formalisms, we show that a graph-theoretic test developed earlier in the context of systems with mass action kinetics, can be applied to reaction systems with arbitrary kinetics. The test, which is easy to implement algorithmically and can sometimes even be decided by observation, rules out the possibility of multiple equilibria in the systems in question.

Key words. Chemical reactions; Injectivity; SR graph; Network structure

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1. Introductory material.

There are many fascinating and increasingly studied questions concerning what conclusions can be drawn about the dynamics of a chemical reaction network based only on the reaction network *structure*, i.e. with limited or absent knowledge of the kinetics. Early work in this direction is exemplified by [1] and [2], with more recent strands including discussions of monotonicity [3, 4, 5], and discussions of injectivity [6, 7, 8]. Although the reaction systems discussed in examples are mostly assumed to have mass action kinetics, an important feature of [4, 8, 5] was that the kinetics was constrained more weakly. The aim of this paper is to extend graph-theoretic results developed in [7] in the context of mass action kinetics to the case of general kinetics. These results, while weaker than the matrix theoretic results in [8], are intuitively pleasing and give rise to conditions which are often easily computable.

A chemical reaction system in which n reactants participate in m reactions has dynamics governed by the ordinary differential equation

$$\dot{x} = Sv(x), \tag{1.1}$$

where $x = [x_1, \ldots, x_n]^T$ is the nonnegative n-vector of reactant concentrations, $v = [v_1, \ldots, v_m]^T$ is the m-vector of reaction rates and S is the $n \times m$ stoichiometric matrix. Arbitrary orderings can be chosen on the sets of substrates and reactions. Further, S is only defined upto an arbitrary re-signing of its columns, equivalent to a switching of the left and right hand sides of a reaction. It is trivial that all results here are independent of the orders chosen on substrates and reactions. We will also confirm below that they are independent of the signing of columns of S. (1.1) defines a dynamical system on the nonnegative orthant in $\mathbb{R}^n$. With the additional assumption that all substances have some inflow (possibly zero) and some outflow proportional to their concentration we get the system

$$\dot{x} = K + Sv(x) - Dx \tag{1.2}$$

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where K is a nonnegative vector representing the inflows and D is a positive diagonal matrix representing the outflow rates. The system has Jacobian $J = SV(x) - D$ where the $m \times n$ matrix $V(x)$ is defined by $V_{ij}(x) \equiv \frac{\partial v_i}{\partial x_j}$. It is notationally convenient to omit the explicit dependence of V on the concentration vector x and write $J = SV - D$.

In [8] and [5] a reaction system was termed **nonautocatalytic** (**NAC** for short) if S and V^T have opposite sign structures in the following sense: $S_{ij}V_{ji} \leq 0$ for all i and j, and $S_{ij} = 0 \Rightarrow V_{ji} = 0$. These conditions are naturally fulfilled provided that no substrate occurs on both sides of a reaction (either with the same or with a changed stoichiometry). Here we refer to systems where no substrates occur on both sides of a reaction, and hence the above two conditions are fulfilled, as **N1C** reaction systems, in order to emphasise that the conditions only rule out *one-step* catalysis.

In [8] it was shown that system 1.2 is injective, and hence incapable of multiple equilibria, provided that the stoichiometric matrix S was "strongly sign determined" (SSD), i.e. all square submatrices of S were either sign nonsingular (as defined in [9] for example) or singular. This led to a characterisation of injectivity based entirely on a computation on the stoichiometric matrix. On the other hand, following theory developed in [6], a labelled multigraph termed the Species-Reaction Graph or **SR graph** was constructed in [7] and used to make claims about the nonexistence of multiple equilibria in systems of chemical reactions with mass-action kinetics. This time, rather than a matrix computation, a certain graph-theoretic condition, sometimes checkable by observation alone without the need for any computation, was shown to be sufficient to guarantee the absence of multiple equilibria. Here we will combine these ideas, and in fact show that the graph-theoretic condition in [7] suffices to guarantee the absence of multiple equilibria for **N1C** systems with arbitrary kinetics.

2. The SR graph. Capturing the structure of the reactions in system 1.1 is a graph termed an SR graph, first introduced in [7]. The SR graph is a bipartite graph with n **substrate vertices**, m **reaction vertices** and an edge between a substrate vertex i and reaction vertex j iff substrate i participates in reaction j. For an **N1C** reaction system there is a one-to-one correspondence between edges in the SR graph and nonzero entries in the stoichiometric matrix S: Clearly $S_{ij} \neq 0$ implies that substrate i participates in reaction j, and with the **N1C** condition it can participate on one side of the reaction only, giving one edge between substrate node i and reaction node j (in the general case multiple edges are allowed). On the other hand, with the **N1C** condition $S_{ij} = 0$ implies that substrate i does not participate in reaction j, and hence that there is no edge connecting substrate i and reaction j.

In this paper, for convenience, we make slight modifications to the labelling of the SR graph in [7], although the fundamental definitions remain unchanged. An edge e_{ij} (where $i \in \{1, \ldots, n\}$ and $j \in \{1, \ldots, m\}$) will be taken to mean an edge between the ith substrate vertex and the jth reaction vertex. Such an edge exists if and only if $S_{ij} \neq 0$. Rather than labelling edge e_{ij} with the complex label associated with substrate i and reaction j as done in [7], we simply give it a sign, so that $\text{sign}(e_{ij}) = \text{sign}(S_{ij})$. Since S is only defined upto an arbitrary signing its columns, the signing of edges in the SR graph is not unique. However, as we will show in Lemma 2.1 after developing some further ideas, all results here are independent of the choice of signing. Whether labelled with the complex labels or signed, the important thing is that the SR graph contains information on whether substrates occur on the same side of a reaction or on opposite sides of a reaction.

Given the one-to-one correspondence

$$S_{ij} \Leftrightarrow e_{ij}$$

when $S_{ij} \neq 0$ it will often be convenient to allow a slight abuse of notation and refer to S_{ij} as an edge in G. Two edges $S_{i_1 j_1}$ and $S_{i_2 j_2}$ share a substrate vertex when $i_1 = i_2$, and they share a reaction vertex when $j_1 = j_2$.

To simplify terminology, given a graph G, we will say that some vertex/edge lies in G meaning that it lies in the vertex/edge-set of G. We will often be interested in subgraphs of G. As we generally define subgraphs E of G by their edge-sets, we will write $E = \{e_1, \dots e_k\}$ where e_i are edges in some graph G to mean that E is the subgraph of G containing exactly these edges and the vertices on which they are incident. A subgraph E defines a second subgraph of G, spanned by the vertex-set of E, which we call G_E. Explicitly, G_E contains only vertices in E, and all edges in G (not necessarily in E) connecting these vertices. The terms **path** and **cycle** will be taken to mean open/closed simple walks in G respectively and are particular examples of subgraphs of G. In the usual way, the size of a subgraph E is the number of edges in E, written $|E|$. When E is a cycle or a path this will also be called the **length** of E. Because of the bipartite nature of SR graphs, all cycles are of even length including alternate substrate and reaction vertices. If two paths or cycles are edge-disjoint they may still share some vertices. When they share no vertices they will be termed vertex-disjoint. A path between a substrate vertex and a reaction vertex is called an **S-to-R path**. S-to-R paths are of odd length.

Cycles in an SR graph have a natural **parity** – they are either odd or even. We use a definition equivalent to that in [7], but using the labelling defined above.

Consider the stoichiometric matrix S and the SR graph G of an **N1C** reaction system (with any fixed choice of signing of S). Let E be any subgraph of G. The sign of an edge e in G has already been defined above, from which we can define the **sign of** E to be

$$\text{sign}(E) = \prod_{e \in E} \text{sign}\,(e)$$

When $|E|$ is even, we define the **parity of** E to be

$$P(E) = (-1)^{|E|/2} \text{sign}(E)$$

Since cycles are always of even length, the parity of a cycle is always defined. A cycle C will be termed an e-cycle if $P(C) = 1$ and an o-cycle if $P(C) = -1$. Note that by these definitions, for an e-cycle C we have

$$\text{sign}(C) = (-1)^{|C|/2},$$

and similarly for an o-cycle C we have

$$\text{sign}(C) = (-1)^{|C|/2-1}.$$

In some situations we will also need to associate the absolute value of S_{ij} with edge e_{ij}. We define the **value** of edge e_{ij} to be $\text{val}(e_{ij}) = |S_{ij}|$, and for a subgraph E,

$$\text{val}(E) = \prod_{e \in E} \text{val}(e)$$

When C is a cycle containing edges $e_1, e_2, \ldots, e_{2r}$ such that e_i and $e_{i+1 \mod 2r}$ are adjacent for each $i = 1, \ldots, 2r$, we can define its **stoichiometry**

$$\text{stoich}(C) = \left| \prod_{i=1}^{r} \text{val}(e_{2i-1}) - \prod_{i=1}^{r} \text{val}(e_{2i}) \right|$$

Note that this definition is independent of the starting point chosen on the cycle. A cycle with $\text{stoich}(C) = 0$ is termed an s-cycle. This definition is equivalent to the definition in [7].

We finish this section with a lemma confirming that re-signing the columns of the stoichiometric matrix does not alter the nature of cycles in an SR graph.

LEMMA 2.1. *Consider an* **N1C** *reaction system with stoichiometric matrix S and SR graph G. Now consider any re-signed version of S, say $S^{'}$ with corresponding SR graph $G^{'}$. Cycles in G are in one-to-one correspondence with those in $G^{'}$. e-cycles (o-cycles) in G correspond to e-cycles (o-cycles) in $G^{'}$. s-cycles in G correspond to s-cycles in $G^{'}$.*

Proof. Ignoring the signs on edges, clearly G and $G^{'}$ are identical graphs, so cycles in G and $G^{'}$ are in one-to-one correspondence. Consider some cycle $C \in G$ and the corresponding cycle $C^{'} \in G^{'}$. Re-signing column j of S means re-signing of all edges incident on reaction node j in $G^{'}$. But clearly C, and hence $C^{'}$ contains either 2 or 0 edges incident on reaction node j. So the re-signing causes no change in $\text{sign}(C)$, i.e. $\text{sign}(C) = \text{sign}(C^{'})$. Finally, re-signing a column does not alter any of the values of edges, leaving $\text{stoich}(C)$ unchanged. Thus the re-signing does not affect the property of a cycle being an s-cycle. □

Finally, it is obvious that S-to-R paths remain so after a re-signing of the raph.

3. Determinants, permutations and cycles. Assume we have some **N1C** reaction system with stoichiometric matrix S and associated SR graph G.

Notation. Given an $n \times m$ matrix S, a submatrix with rows from some set $\gamma \subset \{1, \ldots, n\}$ and columns from some set $\delta \subset \{1, \ldots, m\}$ will be written as $S(\gamma|\delta)$. If $S(\gamma|\delta)$ is square, then it's determinant will be written $S[\gamma|\delta]$. Each submatrix $S(\gamma|\delta)$ corresponds to a subgraph of G which we will term $G(\gamma|delta)$.

Notation. Given a set of the form $\gamma = \{\gamma_1, \gamma_2, \ldots, \gamma_k\}$ we replace the usual brackets with square brackets, i.e. we write $\gamma = [\gamma_1, \gamma_2, \ldots, \gamma_k]$, when it is important to stress that γ is an ordered set.

Consider any square submatrix $S(\gamma|\delta)$ of S with rows and columns indexed by sets $\gamma = \{\gamma_1, \gamma_2, \ldots, \gamma_k\} \subset \{1, \ldots n\}$ and $\delta = \{\delta_1, \delta_2, \ldots, \delta_k\} \subset \{1, \ldots, m\}$ respectively, with $|\gamma| = |\delta| = k$. We always choose $\gamma_1 < \gamma_2 < \ldots < \gamma_k$ and $\delta_1 < \delta_2 < \ldots < \delta_k$. Since δ is an ordered set, any permutation of δ, say α, gives us a term in the expansion of $S[\gamma|\delta]$ of the form $T_\alpha = P(\alpha) S_{\gamma_1 \alpha_1} \cdots S_{\gamma_k \alpha_k}$, where $P(\alpha) = -1$ if α is an odd permutation of δ and $P(\alpha) = 1$ otherwise. Thus terms in the determinant of $S(\gamma|\delta)$ are in one-to-one correspondence with permutations of δ.

Apart from this correspondence, each permutation α (and hence each term T_α) can also be identified with a subgraph in G,

$$E_\alpha = \{S_{\gamma_1 \alpha_1}, \ldots, S_{\gamma_k \alpha_k}\}.$$

Each substrate vertex chosen from γ and each reaction vertex from δ occurs in exactly one edge in E_α. This follows since each member of $\{\gamma_1, \ldots, \gamma_k\}$ occurs exactly once as a first subscript in $S_{\gamma_1 \alpha_1}, \ldots, S_{\gamma_k \alpha_k}$ and similarly each of $\{\delta_1, \ldots, \delta_k\}$ occurs exactly once as a second subscript in this expression. As a result no two edges in E_α share

a vertex. We will refer to a subgraph in $G(\gamma|\delta)$ with these properties as a **term subgraph** in $G(\gamma|\delta)$. Clearly term subgraphs are in one-to-one correspondence with permutations of δ and hence nonzero terms in the expansion of $S[\gamma|\delta]$. They can contain no cycles.

Permutations of a fixed set δ form a group, and so it makes sense to talk about the operations of composition and inversion. Given permutations α, β we can define the permutation $\beta \circ \alpha^{-1}$, i.e. the permutation which takes α regarded as an ordered set to β, regarded as an ordered set. Permutations can be written as products of disjoint cycles. A **nontrivial cycle** will refer to a cycle of length greater than 1. Below we will show that there is a close relationship between cycles as permutations, and cycles in the SR graph. In general the meaning of "cycle" will be clear from the context.

Several key constructions in this paper rely on taking two term subgraphs corresponding to two distinct permutations of δ, say α and β, and looking at the structure of their union $E_\alpha \cup E_\beta$. Consider this union: The ith substrate vertex occurs in exactly one edge in $E_\alpha \cup E_\beta$ if $\alpha_i = \beta_i$, and exactly two edges in $E_\alpha \cup E_\beta$ otherwise. On the other hand, assume $\delta_j = \alpha_i = \beta_k$: If $i = k$, then the jth reaction vertex occurs in exactly one edge in $E_\alpha \cup E_\beta$ while if $i \neq k$ then it occurs in exactly two edges in $E_\alpha \cup E_\beta$. Thus $E_\alpha \cup E_\beta$ contains a set of isolated edges corresponding to trivial cycles in $\beta \circ \alpha^{-1}$, and a set of cycles corresponding to the nontrivial cycles in $\beta \circ \alpha^{-1}$. The explicit construction is carried out in Lemma 3.1 below. All the cycles, trivial or otherwise, are vertex-disjoint (and hence obviously edge-disjoint), since any given vertex has exactly one edge incident on it if it corresponds to a trivial cycle in $\beta \circ \alpha^{-1}$, and exactly two edges incident on it if it corresponds to a nontrivial cycle. In a similar way, given a set of k permutations $\alpha_1, \ldots, \alpha_k$, each vertex in $\cup_{i=1}^{k} E_{\alpha_i}$ has up to k edges incident on it.

Notation. When a permutation is written as a product of cycles, we use round brackets to denote these cycles. To make clear the underlying set we generally include trivial cycles.

Example 1. Let

$$\delta = [\delta_1, \delta_2, \delta_3, \delta_4], \qquad \alpha = [\delta_1, \delta_3, \delta_4, \delta_2], \qquad \beta = [\delta_2, \delta_4, \delta_3, \delta_1]$$

Written as products of disjoint cycles, $\alpha = (\delta_1)(\delta_2, \delta_3, \delta_4)$, $\beta = (\delta_3)(\delta_1, \delta_2, \delta_4)$ and $\beta \circ \alpha^{-1} = (\delta_1, \delta_2)(\delta_3, \delta_4)$. Finally $P(\alpha) = P(\beta) = +1$ and $P(\beta \circ \alpha^{-1}) = P(\beta)P(\alpha) = +1$.

Notation. Given that cycles are the fundamental objects in the theory being developed in this paper, when we have an index k which is known to belong to a set $\{1, \ldots, r\}$, it is always assumed that $k + p$ means $k + p \mod r$. Adopting this convention at the outset avoids lengthy subscripts.

The proof of the next lemma shows the relationship between cycles in permutations and cycles in the graph G, and also begins the process of linking statements about SR graphs and stoichiometric matrices.

LEMMA 3.1. *Consider an* **N1C** *reaction system with stoichiometric matrix S and SR graph G. Assume that there is some submatrix $S(\gamma|\delta)$ and permutations α and β of δ such that T_α and T_β are nonzero terms in $S[\gamma|\delta]$. Then corresponding to each nontrivial cycle in $\beta \circ \alpha^{-1}$ there is a cycle in G. In particular G contains at least one cycle.*

Proof. The two nonzero terms can be written explicitly as:

$$T_\alpha = P(\alpha) S_{\gamma_1 \alpha_1} \cdots S_{\gamma_k \alpha_k}, \qquad T_\beta = P(\beta) S_{\gamma_1 \beta_1} \cdots S_{\gamma_k \beta_k}$$

Write $\beta \circ \alpha^{-1}$ as the product of disjoint cycles. Since $\beta \neq \alpha$ this product contains at least one nontrivial cycle. Consider such a cycle of length r $(1 < r \leq k)$, $(\delta_{b(1)}, \ldots, \delta_{b(r)})$. For each $j \in \{1, \ldots, r\}$ define the index $a(j)$ by $\alpha_{a(j)} = \delta_{b(j)}$. (Note that since α is a permutation, $j_1 \neq j_2 \Leftrightarrow a(j_1) \neq a(j_2)$.) The existence of the cycle means that for each $j \in \{1, \ldots, r\}$, $S_{\gamma_{a(j)}\delta_{b(j)}} \in E_\alpha$ and $S_{\gamma_{a(j)}\delta_{b(j+1)}} \in E_\beta$. Writing these as $\{S_{\gamma_{a(1)}\delta_{b(1)}}, S_{\gamma_{a(1)}\delta_{b(2)}}, S_{\gamma_{a(2)}\delta_{b(2)}}, \ldots S_{\gamma_{a(r)}\delta_{b(r)}}, S_{\gamma_{a(r)}\delta_{b(1)}}\}$ makes it clear that they form a cycle of length $2r$ in G. □

The construction in the previous lemma is best illustrated with an example:

Example 2. Let

$$\begin{aligned}\gamma &= [\gamma_1, \gamma_2, \gamma_3, \gamma_4] \\ \delta &= [\delta_1, \delta_2, \delta_3, \delta_4] \\ \alpha &= [\delta_1, \delta_3, \delta_4, \delta_2] \\ \beta &= [\delta_2, \delta_4, \delta_3, \delta_1]\end{aligned}$$

where $S(\gamma|\delta)$ is a submatrix of a stoichiometric matrix S, $G(\gamma|\delta)$ the associated subgraph, and α and β are permutations of δ associated with nonzero terms in $S[\gamma|\delta]$. α and β define the term subgraphs $E_\alpha = \{S_{\gamma_1\delta_1}, S_{\gamma_2\delta_3}, S_{\gamma_3\delta_4}, S_{\gamma_4\delta_2}\}$ and $E_\beta = \{S_{\gamma_1\delta_2}, S_{\gamma_2\delta_4}, S_{\gamma_3\delta_3}, S_{\gamma_4\delta_1}\}$. From Example 1, since $\beta \circ \alpha^{-1}$ contains nontrivial cycles (δ_1, δ_2) and (δ_3, δ_4), there are two cycles C_1 and C_2 in $E_\alpha \cup E_\beta$. Written out explicitly, these cycles are $C_1 = \{S_{\gamma_1\delta_1}, S_{\gamma_1\delta_2}, S_{\gamma_4\delta_2}, S_{\gamma_4\delta_1}\}$ (corresponding to $a(1) = 1$, $a(2) = 4$, $b(1) = 1$, $b(1) = 2$) and $C_2 = \{S_{\gamma_2\delta_3}, S_{\gamma_2\delta_4}, S_{\gamma_3\delta_4}, S_{\gamma_3\delta_3}\}$ (corresponding to $a(1) = 2$, $a(2) = 3$, $b(1) = 3$, $b(1) = 4$).

The construction in Lemma 3.1 will be used frequently – i.e. given two different permutations α and β of a set δ, cycles in $\beta \circ \alpha^{-1}$ will be used to infer the existence of sets $\{a(j)\}$ and $\{b(j)\}$ and corresponding cycles in the SR graph. When using the construction, for notational brevity we will write a_1 for $\gamma_{a(1)}$ and b_1 for $\delta_{b(1)}$.

So far we have focussed on constructing cycles in an SR graph from pairs of nonzero terms in a determinant. However the reverse is also important – inferring the existence of pairs of nonzero terms in a determinant from structures in a graph. The basic operation which allows us to do this is a particular splitting of a cycle. Any cycle

$$C = \{S_{a_1b_1}, S_{a_1b_2}, S_{a_2b_2}, \ldots, S_{a_rb_r}, S_{a_rb_1}\}$$

can be uniquely partitioned into two vertex-disjoint subgraphs of equal size:

$$C = \{S_{a_1b_1}, S_{a_2b_2}, \ldots, S_{a_rb_r}\} \cup \{S_{a_1b_2}, S_{a_2b_3}, \ldots, S_{a_rb_1}\}. \tag{3.1}$$

We will call this a **disconnecting partition** of C, and each member of the partition is a term subgraph in G_C. With this notion, confirming if a cycle C is an s-cycle now involves:

1. Constructing a disconnecting partition of C into $\{C_1, C_2\}$.
2. Confirming that $\mathrm{val(C_1)} = \mathrm{val(C_2)}$.

4. Preliminary lemmas. With the basic machinery set up above, we are ready to prove some lemmas which will be useful later. The idea of these lemmas is that each of them is quite brief and reusable, so that the proofs of the main results in this paper become simple to state.

The first lemma gives us a basic way of checking whether a permutation, written as a product of disjoint cycles, is even or odd:

LEMMA 4.1. *Consider a permutation α which is as a product of nontrivial cycles from some set $\mathcal{C}$. Let $\theta = \cup_{c\in\mathcal{C}} c$. Then*

$$P(\alpha) = (-1)^{(|\theta|-|\mathcal{C}|)}$$

i.e. the total number of elements in the nontrivial cycles, minus the number of nontrivial cycles is even or odd according to whether α is an even or odd permutation.

Proof. This follows by writing any permutation as the product of disjoint cycles and noting the elementary result that a k-cycle is an even permutation if k is odd and vice versa. □

The next formula gives us a way of deciding on whether two terms in a determinant expansion have the same sign.

LEMMA 4.2. *Consider any square submatrix $S(\gamma|\delta)$ of a stoichiometric matrix S. Consider any two nonzero terms T_α and T_β in the determinant expansion of $S[\gamma|\delta]$. Then*

$$\text{sign}(T_\alpha T_\beta) = (-1)^{2|\theta|-|\mathcal{C}|-|\mathcal{C}_o|} \tag{4.1}$$

where $|\mathcal{C}|$ is the number of cycles in $E_\alpha \cup E_\beta$, $|\mathcal{C}_o|$ is the number of o-cycles in $E_\alpha \cup E_\beta$, and $2|\theta|$ is the number of edges in cycles in $E_\alpha \cup E_\beta$.

Proof. By definition

$$T_\alpha T_\beta = P(\alpha)P(\beta)\prod_{i=1}^{k} S_{\gamma_i\alpha_i}S_{\gamma_i\beta_i}$$

Let θ be the set of indices for which $\alpha_i \neq \beta_i$. We can write

$$T_\alpha T_\beta = P(\alpha)P(\beta)\prod_{i\in\{1,\ldots,k\}\backslash\theta} S_{\gamma_i\alpha_i}S_{\gamma_i\beta_i}\prod_{i\in\theta} S_{\gamma_i\alpha_i}S_{\gamma_i\beta_i}$$

When $i \in \{1,\ldots,k\}\backslash\theta$, $S_{\gamma_i\alpha_i}S_{\gamma_i\beta_i} = S^2_{\gamma_i\alpha_i} > 0$. So

$$\text{sign}(T_\alpha T_\beta) = P(\alpha)P(\beta)\ \text{sign}\left(\prod_{i\in\theta} S_{\gamma_i\alpha_i}S_{\gamma_i\beta_i}\right)$$

Let the set of o-cycles in $\beta\circ\alpha^{-1}$ be $\mathcal{C}_o$ and the set of e-cycles in $\beta\circ\alpha^{-1}$ be $\mathcal{C}_e$. Associate with each $c \in \mathcal{C}_o \cup \mathcal{C}_e$ the corresponding index set $\tilde{c}$, i.e. $i \in \tilde{c} \Leftrightarrow \alpha_i, \beta_i \in c$. Thus corresponding to the sets $\mathcal{C}_o$ and $\mathcal{C}_e$ are index sets $\tilde{\mathcal{C}}_o$ and $\tilde{\mathcal{C}}_e$. Then $\tilde{\mathcal{C}}_o \cup \tilde{\mathcal{C}}_e$ is a partition of θ, and we can define

$$\theta_o = \bigcup_{\tilde{c}\in\tilde{\mathcal{C}}_o}\tilde{c}, \quad \theta_e = \bigcup_{\tilde{c}\in\tilde{\mathcal{C}}_e}\tilde{c} \quad \text{with} \quad |\theta_o| = \sum_{\tilde{c}\in\tilde{\mathcal{C}}_o}|\tilde{c}|, \quad |\theta_e| = \sum_{\tilde{c}\in\tilde{\mathcal{C}}_e}|\tilde{c}|$$

We can write

$$\begin{aligned}\prod_{i\in\theta} S_{\gamma_i,\alpha_i}S_{\gamma_i,\beta_i} &= \left(\prod_{i\in\theta_o} S_{\gamma_i,\alpha_i}S_{\gamma_i,\beta_i}\right)\left(\prod_{i\in\theta_e} S_{\gamma_i,\alpha_i}S_{\gamma_i,\beta_i}\right)\\ &= \left(\prod_{\tilde{c}\in\tilde{\mathcal{C}}_o}\prod_{i\in\tilde{c}} S_{\gamma_i,\alpha_i}S_{\gamma_i,\beta_i}\right)\left(\prod_{\tilde{c}\in\tilde{\mathcal{C}}_e}\prod_{i\in\tilde{c}} S_{\gamma_i,\alpha_i}S_{\gamma_i,\beta_i}\right)\end{aligned}$$

So

$$\begin{aligned}\operatorname{sign}(T_\alpha T_\beta) &= P(\alpha)P(\beta)\left(\prod_{\tilde{c}\in\tilde{\mathcal{C}}_o}\operatorname{sign}\left(\prod_{i\in\tilde{c}}S_{\gamma_i,\alpha_i}S_{\gamma_i,\beta_i}\right)\right)\left(\prod_{\tilde{c}\in\tilde{\mathcal{C}}_e}\operatorname{sign}\left(\prod_{i\in\tilde{c}}S_{\gamma_i,\alpha_i}S_{\gamma_i,\beta_i}\right)\right)\\ &= P(\alpha)P(\beta)\left(\prod_{c\in\mathcal{C}_o}(-1)^{|c|-1}\right)\left(\prod_{c\in\mathcal{C}_e}(-1)^{|c|}\right)\\ &= P(\alpha)P(\beta)(-1)^{|\theta_o|+|\theta_e|-|\mathcal{C}_o|}\end{aligned}$$

Defining $\theta = \theta_o \cup \theta_e$ and $\mathcal{C} = \mathcal{C}_o \cup \mathcal{C}_e$, and applying Lemma 4.1 to $\beta \circ \alpha^{-1}$ gives us that

$$P(\alpha)P(\beta) = P(\beta\circ\alpha^{-1}) = (-1)^{|\theta|-|\mathcal{C}|}$$

so that

$$\operatorname{sign}(T_\alpha T_\beta) = (-1)^{|\theta|-|\mathcal{C}|}(-1)^{|\theta_o|+|\theta_e|-|\mathcal{C}_o|} = (-1)^{2|\theta|-|\mathcal{C}|-|\mathcal{C}_o|}$$

□

A corollary of the previous lemma is that when the intersection of two term subgraphs contains only o-cycles then the two corresponding terms have the same sign.

LEMMA 4.3. *Consider any square submatrix $S(\gamma|\delta)$ of a stoichiometric matrix S. Consider any two nonzero terms T_α and T_β in the determinant expansion of $S[\gamma|\delta]$. If all cycles in $E_\alpha \cup E_\beta$ are o-cycles, then* $\operatorname{sign}(T_\alpha) = \operatorname{sign}(T_\beta)$.

Proof. Applying Equation 4.1 immediately gives

$$\operatorname{sign}(T_\alpha T_\beta) = (-1)^{2|\theta|-|\mathcal{C}|-|\mathcal{C}_o|} = (-1)^{2(|\theta|-|\mathcal{C}|)} = 1$$

where $2|\theta|$ is the number of edges in cycles in $E_\alpha \cup E_\beta$ and $|\mathcal{C}|$ is the number of cycles in $E_\alpha \cup E_\beta$. Thus $\operatorname{sign}(T_\alpha) = \operatorname{sign}(T_\beta)$. □

The next lemma shows that having cycles which are both e-cycles and s-cycles in a graph means that some terms in a determinant expansion sum to zero.

LEMMA 4.4. *Consider a matrix S whose determinant $|S|$ contains two terms T_α and T_β such that $E_\alpha \cup E_\beta$ contains exactly one cycle which is both an e-cycle and an s-cycle. Then $T_\alpha + T_\beta = 0$.*

Proof. By definition

$$T_\alpha + T_\beta = P(\alpha)\prod_{i=1}^{k}S_{\gamma_i\alpha_i} + P(\beta)\prod_{i=1}^{k}S_{\gamma_i\beta_i}$$

As usual, let θ be the set of indices for which $\alpha_i \neq \beta_i$ which are by assumption the indices of vertices in the unique e-cycle C. Defining $C_1 = \{S_{\gamma_i\alpha_i}\}_{i\in\theta}$ and $C_2 = \{S_{\gamma_i\beta_i}\}_{i\in\theta}$ gives us a disconnecting partition of C. We can write

$$\begin{aligned}T_\alpha + T_\beta &= \left(\prod_{i\in\{1,\ldots,k\}\setminus\theta}S_{\gamma_i\alpha_i}\right)\left(P(\alpha)\prod_{i\in\theta}S_{\gamma_i\alpha_i} + P(\beta)\prod_{i\in\theta}S_{\gamma_i\beta_i}\right)\\ &= P(\alpha)\left(\prod_{i\in\{1,\ldots,k\}\setminus\theta}S_{\gamma_i\alpha_i}\right)\left(\prod_{i\in\theta}S_{\gamma_i\alpha_i} + P(\beta\circ\alpha^{-1})\prod_{i\in\theta}S_{\gamma_i\beta_i}\right)\\ &= P(\alpha)\left(\prod_{i\in\{1,\ldots,k\}\setminus\theta}S_{\gamma_i\alpha_i}\right)\left(\operatorname{sign}(C_1)\operatorname{val}(C_1) + P(\beta\circ\alpha^{-1})\operatorname{sign}(C_2)\operatorname{val}(C_2)\right)\end{aligned}$$

$\beta \circ \alpha^{-1}$ can be written as a single cycle of length $|\theta|$, and so from Lemma 4.1, $P(\beta \circ \alpha^{-1}) = (-1)^{|\theta|-1}$. I.e.

$$T_\alpha + T_\beta = P(\alpha) \left(\prod_{i \in \{1,\ldots,k\} \backslash \theta} S_{\gamma_i \alpha_i} \right) \Big(\text{sign}(C_1)\text{val}(C_1) + (-1)^{|\theta|-1} \text{sign}(C_2)\text{val}(C_2) \Big)$$

Since C is an e-cycle we have

$$\text{sign}(C_2)/\text{sign}(C_1) = \text{sign}(C_1)\text{sign}(C_2) = \text{sign}(C) = (-1)^{|\theta|}.$$

Substituting into the expression for $T_\alpha + T_\beta$, we get:

$$T_\alpha + T_\beta = P(\alpha) \left(\prod_{i \in \{1,\ldots,k\} \backslash \theta} S_{\gamma_i \alpha_i} \right) \text{sign}(C_1) \left(\text{val}(C_1) - \text{val}(C_2) \right)$$

However since C is an s-cycle, $\text{val}(C_1) - \text{val}(C_2) = 0$, giving $T_\alpha + T_\beta = 0$. □

The next lemma tells us if a matrix is nonsingular, then not all terms can be paired off in the way carried out in Lemma 4.4.

LEMMA 4.5. *Consider a nonsingular matrix S whose determinant $|S|$ contains two terms T_α and T_β such that $E_\alpha \cup E_\beta$ contains exactly one cycle C which is both an e-cycle and an s-cycle. Define $C_1 = C \cap E_\alpha$ and $C_2 = C \cap E_\beta$ so that $\{C_1, C_2\}$ is a disconnecting partition of C. $|S|$ must contain a term T_σ such that $C_1 \not\subset E_\sigma$ and $C_2 \not\subset E_\sigma$.*

Proof. Assume the contrary. Take any term T_σ in $|S|$. If E_σ contains all the edges from C_1, then we can construct a new term subgraph $E_\tau = (E_\sigma \backslash C_1) \cup C_2$ and a corresponding term T_τ in $|S|$. Alternatively if E_σ contains all the edges from C_2, define $E_\tau = (E_\sigma \backslash C_2) \cup C_1$ with corresponding term T_τ. By construction, $E_\sigma \cup E_\tau$ contains only one cycle which is an e-cycle and an s-cycle and so, by Lemma 4.4, $T_\sigma + T_\tau = 0$. Thus all terms in $|S|$ fall into pairs which sum to zero and S is singular. □

The next lemma tells us a fact which is geometrically obvious about how a term subgraph can intersect a cycle: Either it contains all members in one half of a disconnecting partition of the cycle, or it contains an edge incident on a vertex in the cycle and a vertex outside the cycle.

LEMMA 4.6. *Consider an* **N1C** *reaction system with stoichiometric matrix S and SR graph G. Assume that G contains a cycle C. Let $\{C_1, C_2\}$ be a disconnecting partition of C, and E be any term subgraph in G. If $C_1 \not\subset E$ and $C_2 \not\subset E$, then E contains an edge incident on a substrate vertex in C but not itself in C.*

Proof. Let $|C| = 2k$. Since E is a term subgraph in G, it has an edge incident on each vertex in C. Either some of these are also incident on vertices lying outside C, or E contains k edges in C. In the latter case, either they are all in C_1 or they are all in C_2 or two of them share a vertex. The first two possibilities are ruled out by assumption, and since E is a term subgraph, the third situation is not possible. So E must contain an edge e incident on a vertex in C, but not itself lying in C. If this is a substrate vertex we are done. If it is reaction vertex, then $E \backslash \{e\}$ contains edges incident on k substrate vertices and $k-1$ reaction vertices in C. There must hence be some edge $e' \in E \backslash \{e\}$ incident on a substrate vertex in C but not itself in C. □

Note that in the statement and proof of the above lemma we could exchange "substrate vertex" and "reaction vertex".

Our final preliminary lemma tells us that if a path "slices" an e-cycle in a particular way, this implies the existence of two e-cycles with an S-to-R intersection.

LEMMA 4.7. *Consider an* **N1C** *reaction system with stoichiometric matrix S and SR graph G. Assume that G contains an e-cycle C of length $2r$. Assume that there is a path D in G joining a substrate vertex in C to a reaction vertex in C (i.e. $j, k \in \{1, \ldots, r\}$), but such that D and C are edge-disjoint. Then G contains two e-cycles with an S-to-R intersection.*

Proof. Let $C = \{S_{a_1b_1}, S_{a_1b_2}, S_{a_2b_2}, \ldots, S_{a_rb_r}, S_{a_rb_1}\}$, and let D join substrate vertex a_j to reaction vertex b_k. Decompose C into the two edge-disjoint paths $C = C_1 \cup C_2$ where

$$C_1 = \{S_{a_jb_j}, S_{a_{j+1}b_j}, S_{a_{j+1}b_{j+1}}, \ldots, S_{a_kb_k}\}, \qquad C_2 = \{S_{a_{k+1}b_k}, S_{a_{k+1}b_{k+1}}, \ldots, S_{a_jb_{j-1}}\}$$

Note that $|C_1|$ and $|C_2|$ are both odd since C_1 and C_2 are S-to-R paths. Further, there are two cycles $D_1 = D \cup C_1$ and $D_2 = D \cup C_2$ such that $D_1 \cap D_2 = D$, $D_1 \cap C = C_1$ and $D_2 \cap C = C_2$. The situation is illustrated in Figure 4.1.

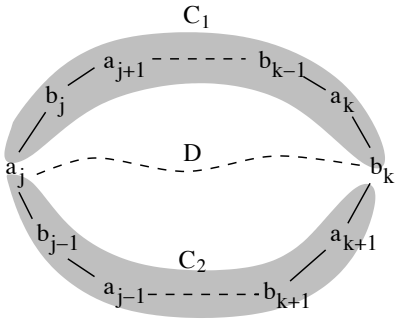

FIG. 4.1. *C_1 and C_2 are S-to-R paths which partition C. D joins a substrate vertex to a reaction vertex in C but does not intersect C.*

We will show that D_1 and D_2 have opposite parity and hence one of them must be an e-cycle. Note that $|C_1| + |C_2| = 2r$, $|D_1| = |D| + |C_1|$ and $|D_2| = |D| + |C_2|$. Subtracting the last two expressions gives $|D_2| - |D_1| = |C_2| - |C_1| = 2(r - |C_1|)$.

The fact that C is an e-cycle means that either

1. r is odd and $\text{sign}(C) = -1$, or
2. r is even and $\text{sign}(C) = +1$.

Case 1. Since $\text{sign}(C) = \text{sign}(C_1)\,\text{sign}(C_2) = -1$:

$$\text{sign}(C_1) = -\text{sign}(C_2)$$

Now r and $|C_1|$ are odd, so $r - |C_1|$ is even, so $|D_2| - |D_1| = 2(r - |C_1|)$ is a multiple of 4, i.e. $(-1)^{|D_2|/2} = (-1)^{|D_1|/2}$. Then

$$P(D_1) = (-1)^{|D_1|/2}\text{sign}(C_1)\text{sign}(D) = (-1)^{|D_2|/2}[-\text{sign}(C_2)]\text{sign}(D) = -P(D_2)$$

So $P(D_2) = -P(D_1)$ and one of D_1 or D_2 must be an e-cycle.

Case 2. This time $\text{sign}(C) = \text{sign}(C_1)\,\text{sign}(C_2) = 1$ so

$$\text{sign}(C_1) = \text{sign}(C_2)$$

Now r is even and $|C_1|$ is odd, so $r - |C_1|$ is odd. As a result $|D_2| - |D_1| = 2(r - |C_1|)$ is not a multiple of 4, and $(-1)^{|D_2|/2} = (-1)^{|D_1|/2+1}$. Again we get $P(D_2) = -P(D_1)$ and one of D_1 or D_2 must be an e-cycle.

In each case one of D_1 or D_2 is an e-cycle. Moreover both D_1 and D_2 intersect C along an S-to-R path (either C_1 or C_2). The result is proved. □

5. Relationship between sign nonsingularity and o-cycles. There is a very simple and elegant relationship between sign nonsingularity in submatrices of the stoichiometric matrix and the non-existence of e-cycles in the SR graph. The results we prove in this section are weaker than our main result in the next section, but have a certain generality to them. At an abstract level, the results have nothing to do with chemical reaction systems and simply describe a relationship between a matrix and an associated digraph. Comments in [9] suggest that these results may be known or suspected, but we have not found a proof in the literature. In any case they are an easy corollary of the previous lemmas.

THEOREM 5.1. *Consider an* **N1C** *reaction system with stoichiometric matrix S and SR graph G. If all cycles in G are o-cycles, then all submatrices of S are either sign nonsingular or identically singular (sign singular).*

Proof. Consider any square submatrix $S(\gamma|\delta)$ of the stoichiometric matrix S with rows and columns indexed in the usual way by ordered sets γ and δ respectively, where $|\gamma| = |\delta| = k$. Consider any permutation α of δ and the corresponding term in $S[\gamma|\delta]$:

$$T_\alpha = P(\alpha) S_{\gamma_1\alpha_1} \cdots S_{\gamma_k\alpha_k}$$

1) If all such terms are zero then the $S(\gamma|\delta)$ is sign singular.

2) If there is a single α for which T_α is nonzero then $S[\gamma|\delta] = T_\alpha$ so clearly $S(\gamma|\delta)$ is sign nonsingular.

3) Consider any pair of nonzero terms in $S[\gamma|\delta]$ corresponding to permutations α and β of δ. Since all cycles are odd, Lemma 4.3 gives us that $\text{sign}(T_\alpha) = \text{sign}(T_\beta)$. Since α and β were arbitrary, this means that all nonzero terms in $S[\gamma|\delta]$ have the same sign. □

The previous theorem has a converse:

THEOREM 5.2. *Consider an* **N1C** *reaction system with stoichiometric matrix S and SR graph G. If all submatrices of S are either sign nonsingular or identically singular (sign singular), then all cycles in G are o-cycles.*

Proof. Assume on the contrary that G has an e-cycle C of length $2r$ including substrate vertices from a set $\gamma = \{\gamma_1, \gamma_2, \ldots, \gamma_r\}$ and reaction vertices from a set $\delta = \{\delta_1, \delta_2, \ldots, \delta_r\}$. There is some permutation α of δ such that C consists of the edges

$$\bigcup_{i\in\{1,\ldots,r\}} \{S_{\gamma_i\alpha_i}, S_{\gamma_i\alpha_{i+1}}\}$$

Setting $\beta_i = \alpha_{i+1}$ defines a permutation β of δ. Clearly $S(\gamma|\delta)$ is not sign singular since

$$T_\alpha = P(\alpha)\prod_{i=1}^{r} S_{\gamma_i\alpha_i}, \quad \text{and} \quad T_\beta = P(\beta)\prod_{i=1}^{r} S_{\gamma_i\beta_i}$$

are nonzero terms in $S[\gamma|\delta]$. Since $E_\alpha \cup E_\beta = C$, and C is an e-cycle, applying Equation 4.1 gives:

$$\text{sign}(T_\alpha T_\beta) = (-1)^{2r-1} = -1$$

Thus T_α and T_β have opposite signs, and $S(\gamma|\delta)$ fails to be sign nonsingular. □

We can state the previous two theorems as a single result:

COROLLARY 5.3. *Consider an* **N1C** *reaction system with stoichiometric matrix S and SR graph G. The following two statements are equivalent:*

1. *All submatrices of S are either sign nonsingular or identically singular.*
2. *All cycles in G are o-cycles.*

Proof. This follows immediately from Theorems 5.1 and 5.2. □

6. A graph theoretic condition ensuring injectivity. Consider the SR graph of a reaction system and define the following condition on it:

Condition (*): All e-cycles in the SR graph are s-cycles, and no two e-cycles have an S-to-R intersection.

In [7] it was shown that Condition (*) was sufficient to ensure injectivity of the Jacobian in any mass action reaction system. For **N1C** reaction systems this means, by results in [8], that Condition (*) is sufficient to ensure that the the stoichiometric matrix of the system is "weakly sign determined" (WSD). However results in [7] and [8] left open the question of whether Condition (*) is sufficient to ensure that the stoichiometric matrix is SSD and hence that the system is injective for essentially arbitrary kinetics. Below we show that the answer is affirmative – Condition (*) is sufficient to ensure that the stoichiometric matrix is SSD. Since SSD implies WSD, as a corollary we reproduce the result that can be inferred from [7] and [8]. We also show by example that Condition (*) is not a necessary condition for the stoichiometric matrix to be SSD.

6.1. Condition (*) ensures that the stoichiometric matrix is SSD. Theorem 6.1 is the main result of this paper: That an **N1C** reaction system whose SR graph fulfils Condition (*) has stoichiometric matrix which is SSD, and is hence, with the outflow condition detailed at the beginning, incapable of multiple equilibria.

THEOREM 6.1. *Consider the SR graph G of an* **N1C** *reaction system with stoichiometric matrix S. Assume that Condition (*) is fulfilled. Then all submatrices of S are either sign nonsingular or singular. In other words S is an SSD matrix.*

Proof. Assume that S is not SSD, i.e. there exists some square submatrix $S(\gamma|\delta)$ of S which is neither sign nonsingular nor singular. Since 1×1 matrices are trivially sign nonsingular or singular, $|\gamma| = |\delta| \geq 2$. The proof will proceed by showing that the corresponding subgraph $G(\gamma|\delta)$ either contains an e-cycle which is an s-cycle or contains two e-cycles which have S-to-R intersection.

Firstly, it is immediate from Theorem 5.1 that $G(\gamma|\delta)$ contains an e-cycle. If $|\gamma| = 2$, then there are exactly two terms in $S[\gamma|\delta]$, i.e. $S_{\gamma_1\delta_1}S_{\gamma_2\delta_2}$ and $-S_{\gamma_1\delta_2}S_{\gamma_2\delta_1}$. Since $S(\gamma|\delta)$ is not sign nonsingular, $\text{sign}(S_{\gamma_1\delta_1}S_{\gamma_2\delta_2}) = \text{sign}(S_{\gamma_1\delta_2}S_{\gamma_2\delta_1})$. Since $S(\gamma|\delta)$ is not singular, $S[\gamma|\delta] = S_{\gamma_1\delta_1}S_{\gamma_2\delta_2} - S_{\gamma_1\delta_2}S_{\gamma_2\delta_1} \neq 0$. Hence, by the definition of an s-cycle, the e-cycle in $G(\gamma|\delta)$ consisting of the edges $\{S_{\gamma_1\delta_1}, S_{\gamma_2\delta_2}, S_{\gamma_1\delta_2}, S_{\gamma_2\delta_1}\}$ is not an s-cycle and condition (*) is violated.

So now assume $|\gamma| \geq 3$. Consider two terms T_α and T_η of opposite sign in $S[\gamma|\delta]$ corresponding to subgraphs E_α and E_η in G. By Lemma 4.3, $E_\alpha \cup E_\eta$ contains an e-cycle (otherwise T_α and T_η would have the same sign). Take an e-cycle $C \subset (E_\alpha \cup E_\eta)$ and construct a disconnecting partition of C into edge-sets $C_1 = C \cap E_\alpha$ and $C_2 = C \cap E_\eta$ according to equation 3.1. Let $|C| = 2k$ for some $2 \leq k \leq |\gamma|$ so that $|C_1| = |C_2| = k$. Define $E_\beta = (E_\alpha \backslash C_1) \cup C_2$. E_α and E_β have been constructed so that their union contains only one cycle (i.e. C). Corresponding to E_α and E_β are nonzero terms T_α and T_β in $S[\gamma|\delta]$. Bearing in mind that there is only one cycle in

$E_\alpha \cup E_\beta$ and this is even, equation 4.1 gives:

$$\text{sign}(T_\alpha T_\beta) = (-1)^{2|\theta_e|-1} = -1$$

There are now two cases to consider. If $T_\alpha + T_\beta \neq 0$, then $\text{val}(E_\alpha) \neq \text{val}(E_\beta)$. I.e. $\text{val}((E_\alpha \backslash C) \cup (E_\alpha \cap C)) \neq \text{val}((E_\beta \backslash C) \cup (E_\beta \cap C))$. Since $E_\alpha \backslash C = E_\beta \backslash C$ we get $\text{val}(E_\alpha \cap C) \neq \text{val}(E_\beta \cap C)$. Finally, since $E_\alpha \cap C$ and $E_\beta \cap C$ together make up a disconnecting partition of C, this means that C is not an s-cycle and we are done.

So assume that $T_\alpha + T_\beta = 0$. Now by Lemma 4.5 we can find a term T_σ in $S[\gamma|\delta]$ such that $C_1 \not\subset E_\sigma$ and $C_2 \not\subset E_\sigma$. As a result, by Lemma 4.6, E_σ contains some edge incident on a substrate vertex in C, but not itself in C. In terms of the permutations, there is an index q such that vertices $\alpha_q, \beta_q \in C$, but $\sigma_q \neq \alpha_q$ and $\sigma_q \neq \beta_q$.

Consider $\sigma \circ \alpha^{-1}$ as the product of disjoint cycles. Since $\sigma_q \neq \alpha_q$, one of these cycles involves σ_q. Further, since $\sigma_q \neq \beta_q$, the corresponding cycle in G is distinct from C but intersects C at substrate vertex γ_q. Let this cycle be termed $C_{\alpha\sigma}$ (to remind us that it is composed of edges from E_α and E_σ) and have length $2r$ ($1 < r \leq |\gamma|$). In the usual way, we can follow $C_{\alpha\sigma}$: There are distinct indices $a_1, \ldots, a_r \in \gamma$ and $b_1, \ldots, b_r \in \delta$ such that the edges $S_{a_j b_j}$ occur in E_α and $S_{a_j, b_{j+1}}$ occur in E_σ. Assume (without loss of generality, i.e. by reordering the sets a_i and b_i if necessary) that $a_1 = \gamma_q$ and $b_2 = \sigma_q$, so that $S_{a_1 b_2}$ is the edge incident on a substrate vertex in C, but not itself lying in C.

Follow the cycle $C_{\alpha\sigma}$ starting at $S_{a_1 b_2} \in T_\sigma$ i.e.

$$S_{a_1 b_2} \in T_\sigma,\ S_{a_2 b_2} \in T_\alpha,\ S_{a_2 b_3} \in T_\sigma,\ \ldots$$

Since this is a cycle, eventually some vertex from the sequence of alternating reaction and substrate vertices indexed by $(b_2, a_2, b_3, \ldots)$ must be a vertex in C. But this cannot first happen at a substrate vertex. Suppose the contrary and substrate vertex a_j is in C, while reaction vertex b_j is not. Since edge S_{a_j, b_j} is in T_α, but not in C, this implies that substrate vertex a_j has three edges from $T_\alpha \cup T_\beta$ incident on it – the two edges in C along with the edge S_{a_j, b_j}. But this is impossible from the discussion in Section 3. This means that from the vertex sequence $(b_2, a_2, b_3, \ldots)$ the first vertex to lie in C must be a reaction vertex. Let this vertex be b_j ($j = 2$ is possible). Define the path $D \equiv \{S_{a_1 b_2}, S_{a_2 b_2}, S_{a_2 b_3}, \ldots, S_{a_{j-1} b_j}\}$. D is an S-to-R path starting and terminating at vertices in C but edge-disjoint from C.

Now applying Lemma 4.7 shows that there are two e-cycles in G (one of which is C and one of which is made up of D and some part of C) which have an S-to-R intersection. Thus if the stoichiometric matrix S is not SSD, then the associated graph G necessarily fails Condition (*). The result is proved. □

6.2. Condition (*) is not necessary for SSD (or WSD). We construct a somewhat artificial example to show that the condition on the SR graph is not necessary to give a system with an SSD matrix. Take the system of three reactions

$$D \rightleftharpoons A + B + C,$$
$$E \rightleftharpoons A + B + C,$$
$$F \rightleftharpoons A + B + C.$$

This system has stoichiometric matrix

$$S = \begin{bmatrix} 1 & 1 & 1 \\ 1 & 1 & 1 \\ 1 & 1 & 1 \\ -1 & 0 & 0 \\ 0 & -1 & 0 \\ 0 & 0 & -1 \end{bmatrix}$$

which can quickly be computed to be SSD, implying that the system with inflows and outflows forbids multiple equilibria. The SR graph for the system is shown in Figure 6.1. Although all e-cycles are s-cycles, there are e-cycles with an S-to-R intersection.

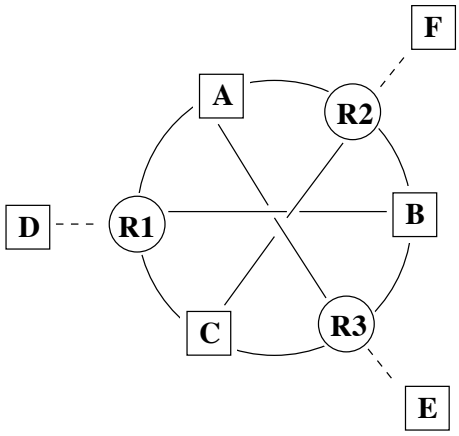

FIG. 6.1. *The SR graph of the system of three reactions shown above. Positive edges are bold lines while negative edges are dashed lines. There are a number of e-cycles with S-to-R intersection, for example the cycles* $A-R2-B-R3$ *and* $A-R1-C-R3$ *intersect along the S-to-R path* $A-R3$.

Thus Condition (*) is not necessary for injectivity in the case of a general system of **N1C** reaction. Since the condition that S is SSD is stronger than the condition that S is WSD, clearly Condition (*) is not necessary for injectivity in the case of a system of mass action reactions.

7. Conclusions. We have proved a number of results for the class of **N1C** reaction systems with arbitrary kinetics. Given such a system with stoichiometric matrix S and SR graph G, the two key results can be summarised by:

1. All cycles in G are o-cycles iff all submatrices of S are either sign nonsingular or identically singular (Corollary 5.3).
2. Condition (*) on G is sufficient, but not necessary, to guarantee that S is SSD (Theorem 6.1 and the counterexample in Section 6.2).

With the inflow and outflow conditions in Equation 1.2, these translate immediately into statements about the nonexistence of multiple equilibria. It remains open whether there is a condition on G which is equivalent to S being SSD. As a step towards this, it would be interesting to characterise those systems which fail Condition (*) but have SSD stoichiometric matrices. Finally, there are natural extensions of this work in the situation where the **N1C** condition is weakened to allow one-step catalysis, provided that any substrate which occurs on both sides of a reaction occurs with the same stoichiometry on both sides. These themes will be pursued in future work.

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