

# *P* MATRIX PROPERTIES, INJECTIVITY AND STABILITY IN CHEMICAL REACTION SYSTEMS

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**Abstract.** In this paper we examine matrices which arise naturally as Jacobians in chemical dynamics. We are particularly interested in when these Jacobians are *P* matrices (up to a sign change), ensuring certain bounds on their eigenvalues, precluding certain behaviour such as multiple equilibria, and sometimes implying stability. We first explore reaction systems and derive results which provide a deep connection between system structure and the *P* matrix property. We then examine a class of systems consisting of reactions coupled to an external rate-dependent negative feedback process, and characterise conditions which ensure the *P* matrix property survives the negative feedback. The techniques presented are applied to examples published in the mathematical and biological literature.

**Key words.** Chemical reactions; *P* matrices; Injectivity; Stability; Mass action

**AMS subject classifications.** 80A30; 15A48; 34D30

**1. Introduction.** In this paper we will study chemical reaction systems, and systems derived from these. Chemical reaction systems have Jacobians with more structure than those of arbitrary dynamical systems. Under mild assumptions we derive a condition on the reaction structure which ensures a reaction system has Jacobians in a particular class,  $P_0^{(-)}$  matrices, to be defined below. This condition is algorithmically easy to check, and immediately implies the absence of multiple equilibria as long as there are appropriate outflow conditions. A weaker condition is then derived specifically for mass action reaction systems, which ensures that they have Jacobians in this class, and hence, under appropriate outflow conditions, cannot have multiple equilibria. These conditions are shown to be not only sufficient to preclude multiple equilibria, but also necessary to ensure that the Jacobians can never be singular. Finally a class of systems of particular importance in biochemistry is examined. These systems involve reactions interacting with some external quantity giving rise to a negative feedback process. Necessary and sufficient conditions are derived which ensure that the *P* matrix properties of the system without feedback persist with the feedback.

**2. Basic material.** We start with some basic definitions and observations.

**2.1. Chemical reaction systems.** A chemical reaction system in which  $n$  reactants participate in  $m$  reactions has dynamics governed by the ordinary differential equation

$$\dot{x} = Sv(x) \tag{2.1}$$

Here  $x = [x_1, \dots, x_n]^T$  is the nonnegative  $n$ -vector of reactant concentrations,  $v = [v_1, \dots, v_m]^T$  is the  $m$ -vector of reaction rates and  $S$  is the  $n \times m$  stoichiometric matrix. (2.1) defines a dynamical system on  $\mathbb{R}_+^n$  (the nonnegative orthant in  $\mathbb{R}^n$ ). The entries in  $S$  are constants – generally integers – with  $|S_{ij}|$  describing how many molecules of substrate  $i$  are involved in reaction  $j$ . The sign of  $S_{ij}$  reflects an arbitrary choice

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<sup>§</sup>Funded by an EPSRC/MRC grant to the MIAS IRC (Grant Ref: GR/N14248/01)

of direction for the reaction, with no implication of reversibility or irreversibility. We will generally assume that substrates only occur on one side of a reaction (more on this later) and if  $S_{ij} < 0$  we will say that substrate  $i$  occurs on the “left hand side” of reaction  $j$ , and on the “right hand side” if  $S_{ij} > 0$ .

The same form (2.1) can represent either a closed reaction system where there is no inflow or outflow of reactants, or an open system. For an open system we simply allow some of the reactions to have empty left or right hand sides. We will refer to reactions not involving any inflow or outflow as “true” reactions.  $S$  describes a linear mapping between the reaction rates and the time derivatives of the concentrations, and any steady states of (2.1) must correspond to reaction rates lying in the kernel of  $S$ . Thus a nontrivial kernel means that there are steady states corresponding to nonzero reaction rates.

The  $m \times n$  matrix  $V(x)$  defined by  $V_{ij}(x) \equiv \frac{\partial v_i}{\partial x_j}$  describes the dependence of the reaction rates on the concentrations. For later notational convenience we will write  $V$  instead of  $V(x)$ . The Jacobian of (2.1) is then just  $SV$ .

To make progress, we need to narrow the class of reactions a little. We call a reaction system **nonautocatalytic** (**NAC** for short) if the stoichiometric matrix  $S$  and the matrix  $V^T$  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 assumptions are quite general – they mean that if a substrate is used up (created) in a reaction, then increasing the concentration of this substrate, while holding all others constant, cannot cause the reaction rate to decrease (increase). Further, if a substrate does not participate in a reaction, then it is not allowed to influence the reaction rate. As we allow  $S_{ij}V_{ji} = 0$ , even when  $S_{ij} \neq 0$ , irreversible reactions are implicitly allowed by this definition.

The assumption that the system is NAC holds for mass action systems, Michaelis-Menten systems, etc, provided that a reactant only occurs on one side of a reaction. It is possible to violate this condition, for example with reactions such as  $A + B \rightleftharpoons 2A$ , where perhaps for small concentrations of  $A$  net flux is to the right while for large concentrations it is to the left. Sometimes, in practice, such reactions actually represent the amalgamation of several NAC reactions. For example, the above system might actually represent  $A + B \rightleftharpoons C$ ,  $C \rightleftharpoons 2A$ , where  $C$  is some short-lived intermediate complex. If a reaction can be rewritten in this way then it becomes amenable to the analysis presented here.

Most results in this paper are independent of the functional forms chosen for the reaction dynamics, apart from the assumption that reactions are NAC, as just described. However, some of the results which motivated this work are those of Craciun and Feinberg [5, 6] on the possibility of multiple equilibria in **mass action systems**, and the techniques they present to deduce the absence of multiple equilibria from the reaction network structure alone. Since we have included some results on mass action systems we define these here. Let  $\nu_j$  be the set of indices of the reactants on the left hand side of the  $j$ th reaction, and  $\rho_j$  be the set of indices of the reactants on the right hand side of the  $j$ th reaction. Further let  $L_{ij}$  be the number of molecules of substrate  $i$  occurring on the left hand side of the  $j$ th reaction, and  $R_{ij}$  be the number of molecules of substrate  $i$  occurring on the right hand side of the  $j$ th reaction. Then, for a mass action system, the reaction rate  $v_j$  for the  $j$ th reaction takes the form

$$v_j = k_j \prod_{i \in \nu_j} x_i^{L_{ij}} - k_{-j} \prod_{i \in \rho_j} x_i^{R_{ij}}$$

where  $k_j$  and  $k_{-j}$  are nonnegative constants, known as the forward and backward

rate constant for the  $j$ th reaction. When the reaction is NAC, this can be rewritten in terms of entries in the stoichiometric matrix to get:

$$v_j = k_j \prod_{i \in \nu_j} x_i^{-S_{ij}} - k_{-j} \prod_{i \in \rho_j} x_i^{S_{ij}}$$

We can clearly write a single reversible reaction as two irreversible reactions.

**2.2.  $P$  matrices and related classes.** For some matrix  $A$ ,  $A(\alpha|\gamma)$  will refer to the submatrix of  $A$  with rows indexed by the set  $\alpha$  and columns indexed by the set  $\gamma$ . A **principal submatrix** of  $A$  is a submatrix containing columns and rows from the same index set, i.e. of the form  $A(\alpha|\alpha)$ , which we will abbreviate to  $A(\alpha)$ . A **minor** is the determinant of a square submatrix. If  $A(\alpha|\gamma)$  is a square submatrix of  $A$  (i.e.  $|\alpha| = |\gamma|$ ), then  $A[\alpha|\gamma]$  will refer to the corresponding minor, i.e.  $A[\alpha|\gamma] = \det(A(\alpha|\gamma))$ . A **principal minor** of a matrix is the determinant of a principal submatrix.  $A[\alpha]$  will refer to principal minor corresponding to submatrix  $A(\alpha)$ .

$P$  matrices are square matrices all of whose principal minors are positive. They are nonsingular and their eigenvalues are excluded from a certain wedge around the negative real axis [15]. If  $-A$  is a  $P$  matrix, then we will say that  $A$  is a  $P^{(-)}$  matrix. These matrices were originally called  $N-P$  matrices in [17]. Throughout this paper, when  $A$  is a matrix such that  $-A$  belongs to some class  $\mathcal{C}$ , then we will say that  $A$  belongs to the class  $\mathcal{C}^{(-)}$ . If  $A$  is a  $P^{(-)}$  matrix, this means that each  $k \times k$  principal minor of  $A$  has sign  $(-1)^k$ . The problem of checking whether a given matrix is a  $P$  matrix is in general N-P hard [19].

Another important characterisation of  $P$  matrices is that a matrix  $A$  is a  $P$  matrix iff for any nonzero vector  $y$ , there is some index  $i$  such that  $y_i(Ay)_i > 0$  [3]. It follows immediately that a matrix  $A$  is a  $P^{(-)}$  matrix iff for any nonzero vector  $y$ , there is some index  $i$  such that  $y_i(Ay)_i < 0$ . In other words a  $P^{(-)}$  matrix maps each nonzero vector  $y$  out of any orthants in which it lies. (As orthants share boundaries,  $y$  may lie in several orthants at once.)

$P$  matrices contain other important classes of matrices, such as positive definite matrices and also so-called nonsingular  $M$  matrices. As these will be mentioned again later we define them here.  $Z$  matrices are square matrices all of whose off-diagonal entries are less than or equal to zero. Nonsingular  $M$  matrices are precisely those matrices which are both  $Z$  matrices and  $P$  matrices, i.e. matrices whose off-diagonal elements are nonpositive and all of whose principal minors are positive. Using the notational convention defined above,  $M^{(-)}$  matrices are matrices which are both  $Z^{(-)}$  matrices and  $P^{(-)}$  matrices.

A related class of matrices are  $P_0$  matrices consisting of the closure of the set of  $P$  matrices. These are matrices all of whose principal minors are nonnegative [14]. Similarly  $A$  is a  $P_0^{(-)}$  matrix if  $-A$  is a  $P_0$  matrix. A matrix  $A$  is a  $P_0$  matrix iff for any nonzero vector  $y$ , there is some index  $i$  such that  $y_i(Ay)_i \geq 0$ , and similarly it is a  $P_0^{(-)}$  matrix iff for any nonzero vector  $y$ , there is some index  $i$  such that  $y_i(Ay)_i \leq 0$ . By definition  $P_0$  and  $P_0^{(-)}$  matrices can be singular.

**2.3. Implications of a  $P^{(-)}$  Jacobian: Injectivity and Stability.** In the work of Craciun and Feinberg [5, 6] global injectivity, and hence the absence of multiple equilibria, follow from the nonsingularity of the Jacobian. This is not true for general functions – it is well known that nonsingularity of the Jacobian alone does not imply global injectivity of arbitrary polynomial functions [18]. In this direction there are several results connecting properties of functions with injectivity. A well known

theorem of Hadamard [12] states that nonsingularity of the Jacobian ensures global injectivity provided that the function is **proper** – i.e. the preimage of any compact set is compact. Recent elegant work such as that in [9] and [20] provides conditions (not all spectral) which ensure that a function is globally injective.

Regarding  $P$  matrices, there is a result stating that if the Jacobian of a function is a  $P$  matrix (or equivalently a  $P^{(-)}$  matrix), this guarantees injectivity of the function on any rectangular region of  $\mathbb{R}^n$  [10]. The result for all of  $\mathbb{R}^n$  also follows from the geometric fact mentioned in Section 2.2 that  $P^{(-)}$  matrices map vectors out of the orthants in which they lie. Thus for a fixed nonzero vector  $y$ , every  $P^{(-)}$  matrix must rotate  $y$  by at least some angle  $\theta > \theta_y > 0$  – where  $\theta_y$  is the infimum of the angular distance from  $y$  to an orthant to which  $y$  does not belong; thus for any unit vector  $y$  and any set of  $P^{(-)}$  matrices  $A(x)$ ,

$$\sup_x \left\langle y, \frac{A(x)y}{|A(x)y|} \right\rangle < \cos \theta_y < 1$$

From Theorem 2 in [20], this condition on the Jacobian guarantees global injectivity of the function.

[10] also contains the following strengthened result which weakens the condition needed for injectivity: If the Jacobian of a function is a nonsingular  $P_0$  matrix (termed a “weak  $P$  matrix” in this reference), this guarantees injectivity of the function on any rectangular region of  $\mathbb{R}^n$ . The result obviously holds for a nonsingular  $P_0^{(-)}$  matrix as well.

While the ruling out of multiple equilibria is the first and perhaps most important consequence of finding that a particular dynamical system gives rise to  $P^{(-)}$  matrix Jacobians, sometimes stronger conclusions can be drawn. In particular, if a matrix  $J$  is a  $P^{(-)}$  matrix, then Hurwitz stability of  $J$  may follow from additional observations. We list three of these:

1. If  $J$  is similar to a symmetric matrix, and thus has real eigenvalues, then it must be Hurwitz stable,
2. If all off-diagonal elements of  $J$  are nonnegative, then it is in fact a nonsingular  $M^{(-)}$  matrix [3] and hence Hurwitz stable,
3. A weaker condition is when  $J$  is “sign symmetric” meaning that all symmetrically placed pairs of minors have the same sign: Then it is stable because sign-symmetric  $P^{(-)}$  matrices are Hurwitz stable [14]. Certain physical assumptions can give rise to Jacobians which are sign-symmetric.

In this paper we will refer to a reaction system whose Jacobians are always  $P^{(-)}$  matrices as  $P^{(-)}$  systems and ones whose Jacobians are always  $P_0^{(-)}$  matrices as  $P_0^{(-)}$  systems.

**2.4. Rate dependent negative feedback processes.** The assumption that a reaction is NAC means, roughly speaking, that every substrate interacts with the reactions in which it participates in the following way: if it is produced by a reaction, then it inhibits the reaction. If it is used up by a reaction, then it activates the reaction. Physically any scalar quantity  $\psi$  which behaves like this participates in **rate-dependent negative feedback**, and adding such a quantity to a system adds a rate-dependent negative feedback process to the system. Although  $\psi$  might be the concentration of a chemical, this need not be the case – for example  $\psi$  may take negative values. In an example of biological importance discussed in [1] and used to illustrate our results below,  $\psi$  is in fact a chemical and electrical **gradient** with which

some of the reactions interact because they pump material across a membrane. This is a frequent occurrence in biochemistry: Quite generally where reactions involve the build-up of gradients between compartments, we get such systems.

Adding a rate-dependent negative feedback process, whether a reactant or not, to a reaction system involves choosing two vectors  $x_1, x_2 \in \mathbb{R}^m$  and adding a row  $x_1^T$  to  $S$  and a column  $x_2$  to  $V$  to get augmented versions,  $S_{aug}$  and  $V_{aug}$ , of these matrices. The negative feedback assumption means that  $x_1$  and  $x_2$  lie in opposite orthants so that  $x_{1,i}x_{2,i} \leq 0$ . In general, if  $x_{1,i} = 0$ , then  $x_{2,i} = 0$ , but it is convenient to ignore this and ask the more general question: Given that  $SV$  is a  $P^{(-)}$  matrix, when will  $S_{aug}V_{aug}$  be a  $P_0^{(-)}$  matrix for all possible  $x_1, x_2 \in \mathbb{R}^m$  lying in (specified) opposite orthants? Given particular orthants, it is possible to state necessary and sufficient conditions on  $S$  and  $V$  which answer this question, and with appropriate outflow conditions to replace  $P_0^{(-)}$  with  $P^{(-)}$  in the above statement.

**3.  $P^{(-)}$  matrices and general reaction systems.** We now examine the close connection between  $P^{(-)}$  matrices and reaction systems of the form (2.1). After some preliminaries we present a structural result giving a sufficient condition on the stoichiometric matrix  $S$  which will ensure that the Jacobian will be a  $P_0^{(-)}$  matrix. In a sense to be made precise this condition is also a necessary condition.

We need some definitions first: A real matrix  $S$  determines a **qualitative class** [4] of all matrices with the same sign pattern as  $S$ , which we will refer to as  $\mathcal{Q}(S)$ . It is helpful to think of  $\mathcal{Q}(S)$  as a matrix with entries consisting of zeros and variables of fixed sign, and  $\det(\mathcal{Q}(S))$  is then a polynomial in these variables. If  $\det(\mathcal{Q}(S))$  is not identically zero, then it is a sum of monomials, each of which is either positive or negative. It also makes sense to refer to  $\overline{\mathcal{Q}(S)}$ , as the closure of  $\mathcal{Q}(S)$  (regarded as a set of matrices), and  $\det(\overline{\mathcal{Q}(S)})$ , as the same polynomial as  $\det(\mathcal{Q}(S))$  with variables now allowed to take the value zero. In this terminology a reaction system is NAC if  $V \in \overline{\mathcal{Q}(-S^T)}$ .

A square matrix is **sign-nonsingular**, or **SNS** [4] if the sign of its determinant is nonzero, and can be determined from the signs of its entries. In other words if the sign of the determinant is the same for every matrix in its qualitative class. For example any 2-by-2 matrix with a single negative, positive or zero entry is SNS. On the other hand a 2-by-2 matrix with two positive and two negative entries is not SNS. If any square matrix  $T$  is SNS, then it makes sense to talk about  $\text{sign}(\det(\mathcal{Q}(T)))$ .

A (not necessarily square) matrix  $S$  will be termed **strongly sign determined** (SSD for short) if all square submatrices of  $S$  are either SNS or singular. SSD matrices intersect various classes of matrices discussed in [4], for example the so-called totally  $L$ -matrices and the  $S^2NS$  matrices, and the SSD property is algorithmically quick and easy to check. Some results concerning SSD matrices are collected in Appendix A. These properties show among other things that alternative notational choices in chemical dynamics – for example the choice to represent one reversible reaction as two irreversible ones, which side of a reaction to consider as the left hand side, how to order the set of substrates, or how to order the set of reactions – never change whether the stoichiometric matrix is SSD or not.

For the proofs which follow in this section, it is convenient to set up the following notational conventions.  $S$  will always refer to some particular, but unspecified stoichiometric matrix. Since we are interested in NAC, but otherwise unspecified, reaction systems in this section, given a matrix  $S$ , it is convenient for  $V$  to refer to the closure of a whole class  $\overline{\mathcal{Q}(-S^T)}$ . Similarly  $V(\gamma|\alpha)$  will refer to  $\overline{\mathcal{Q}(-S(\alpha|\gamma))}$  and  $V[\gamma|\alpha]$  will refer to the polynomial  $\det(\overline{\mathcal{Q}(-S(\alpha|\gamma))})$ . If we refer to a “choice of  $V$ ”,

then this means some particular matrix in  $\overline{\mathcal{Q}(-S^T)}$ . Objects defined as products will take the appropriate meanings, for example an object such as  $S[\alpha|\gamma]V[\gamma|\alpha]$  is again a polynomial.

It helps to note a few obvious, but important, **preliminaries** about SSD matrices:

1. If a matrix  $S$  is SSD, then so is  $-S$ . In particular, given any square submatrix  $S(\alpha|\gamma)$  which is SNS

$$\text{sign}(\det(-S(\alpha|\gamma))) = (-1)^{|\alpha|} \text{sign}(\det(S(\alpha|\gamma)))$$

On the other hand if  $S(\alpha|\gamma)$  is singular, then so is  $-S(\alpha|\gamma)$ .

2. If a stoichiometric matrix  $S$  is not SSD, then there is some square submatrix  $S(\alpha|\gamma)$  such that  $\det(\mathcal{Q}(S(\alpha|\gamma)))$  – and hence  $V[\gamma|\alpha]$  – contains both a positive and a negative term.

We can now state our first theorem.

**THEOREM 3.1.** *If the stoichiometric matrix  $S$  of a NAC reaction system is SSD, then the Jacobian  $J = SV$  is a  $P_0^{(-)}$  matrix.*

*Proof.* Let  $J[\alpha]$  be the principal minor of  $J$  corresponding to the submatrix with rows and columns indexed by a set  $\alpha \subset \{1, \dots, n\}$ . By the Cauchy-Binet formula ([11] for example) we get

$$J[\alpha] = (SV)[\alpha] = \sum_{\substack{\gamma \subset \{1, \dots, m\} \\ |\gamma| = |\alpha|}} S[\alpha|\gamma]V[\gamma|\alpha]$$

The sum is over all subsets of  $\{1, \dots, m\}$  of size  $|\alpha|$ , if any such subsets exist. Since the reaction system is NAC and  $S$  is SSD, for each  $\gamma$ , either  $S[\alpha|\gamma]$  is zero or  $S(\alpha|\gamma)$  is SNS in which case

$$\text{sign}(V[\gamma|\alpha]) = (-1)^{|\alpha|} \text{sign}(S[\alpha|\gamma])$$

by preliminary 1 above. So  $J[\alpha]$  is a sum of terms each of which is either zero or has sign  $(-1)^{|\alpha|}$  and thus either  $J[\alpha] = 0$  or  $\text{sign}(J[\alpha]) = (-1)^{|\alpha|}$ .  $\square$

A natural question which arises is whether there is any kind of converse to Theorem 3.1, or equivalently whether there could be a weaker condition on the stoichiometric matrix which would still always ensure a  $P_0^{(-)}$  Jacobian. The answer, provided in the next theorem, is that there is no weaker condition guaranteeing a  $P_0^{(-)}$  Jacobian.

**THEOREM 3.2.** *Assume that the stoichiometric matrix  $S$  of a NAC system is not SSD. Then there is some choice of  $V$  for which  $SV$  is not a  $P_0^{(-)}$  matrix.*

*Proof.* Since  $S$  is not SSD, there are sets  $\alpha_0 \subset \{1, \dots, n\}$ ,  $\gamma_0 \subset \{1, \dots, m\}$  with  $|\alpha_0| = |\gamma_0|$ , such that  $S(\alpha_0|\gamma_0)$  is neither SNS nor singular. Consider

$$J[\alpha_0] = \sum_{\substack{\gamma \subset \{1, \dots, m\} \\ |\gamma| = |\alpha_0|}} S[\alpha_0|\gamma]V[\gamma|\alpha_0]$$

Since  $S(\alpha_0|\gamma_0)$  is not SNS,  $V[\gamma_0|\alpha_0]$  contains a term  $t$  such that  $S[\alpha_0|\gamma_0]t$  is of the “wrong” sign:  $(-1)^{|\alpha_0|+1}$ . This follows because, as noted in preliminary 2 earlier, since  $S(\alpha_0|\gamma_0)$  is not SNS,  $V[\gamma_0|\alpha_0]$  contains both positive and negative terms. But  $t$  is just a term in the determinant of a submatrix of  $V$ , i.e. a product of entries of  $V$ .

Set all entries in  $V$  which do not figure in  $t$  to 0. Since determinants are homogeneous polynomials in the entries of a matrix, and no entry has power higher than 1, all terms in  $J[\alpha_0]$  other than  $S[\alpha_0|\gamma_0]t$  become zero, so that  $J[\alpha_0] = S[\alpha_0|\gamma_0]t$  which has sign  $(-1)^{|\alpha_0|+1}$ . Hence  $J$  is not a  $P_0^{(-)}$  matrix. By continuity, since the set of matrices which are not  $P_0^{(-)}$  matrices is open, the argument still holds if entries in  $V$  not occurring in  $t$  are sufficiently small but non-zero.  $\square$

Incidentally we could phrase the above two results together as the following corollary possibly of broad interest:

**COROLLARY 3.3.** *Consider an  $n \times m$  matrix  $A$ . Then  $A$  is SSD if and only if  $AB$  is a  $P_0$  matrix for every  $m \times n$  matrix  $B$  which satisfies  $A_{ij}B_{ji} \geq 0$  and  $A_{ij} = 0 \Rightarrow B_{ji} = 0$ .*

*Proof.* The proof is immediate from the previous two results.  $\square$

Although the discussion so far has been of  $P_0^{(-)}$  matrices, it is clear from the proofs that if, in addition to  $S$  being SSD, for  $\alpha = \{1, \dots, n\}$  there is some  $\gamma$  such that  $S[\alpha|\gamma]$  and  $V[\gamma|\alpha]$  are both nonzero then  $J$  is in fact nonsingular  $P_0^{(-)}$ , and the function is injective. And if, for every  $\alpha$ , there is some  $\gamma$  such that  $S[\alpha|\gamma]$  and  $V[\gamma|\alpha]$  are both nonzero, then  $J$  is in fact a  $P^{(-)}$  matrix (and injective). This often arises in practice because there are inflow and outflow processes contributing terms on the diagonal of  $SV$ . For example, Continuous Flow Stirred Tank Reactors (CFSTRs) as presented in [5] have properties which ensure that for non-zero flow rate any Jacobian which is a  $P_0^{(-)}$  matrix is in fact a  $P^{(-)}$  matrix. Using  $S$  to refer to the stoichiometric matrix of the “true” reactions in a CFSTR (excluding the inflow and outflow processes), a CFSTR system can be written:

$$\dot{x} = q(x_{in} - x) + Sv(x) \quad (3.1)$$

where  $q$  is a positive scalar representing the flow rate through the reactor and  $x_{in}$  is a nonnegative vector representing the “feed” concentration. We have:

**THEOREM 3.4.** *Assume all the reactions in a CFSTR are NAC. If the stoichiometric matrix  $S$  is SSD then the Jacobian of the system is a  $P^{(-)}$  matrix.*

*Proof.* The full stoichiometric matrix  $S_f$  of a CFSTR system can be written in block form

$$S_f = [S | -I_n]$$

where  $S$  is the matrix of true reactions and  $I_n$  is the  $n \times n$  unit matrix. Similarly define  $V_f$  by

$$V_f = \begin{bmatrix} V \\ qI_n \end{bmatrix}$$

The Jacobian of the system is

$$J \equiv S_f V_f = -qI + SV$$

where  $I$  is the identity matrix. Since the reactions are NAC and  $S$  is SSD this means, by Theorem 3.1, that  $SV$  is a  $P_0^{(-)}$  matrix. As mentioned in Section 2.2, a matrix  $A$  is

a  $P_0$  matrix iff for any nonzero vector  $y$ , there is some index  $i$  such that  $y_i(Ay)_i \geq 0$ , and similarly it is a  $P$  matrix iff for any nonzero vector  $y$ , there is some index  $i$  such that  $y_i(Ay)_i > 0$ . So any  $P_0$  matrix plus a positive diagonal matrix is a  $P$  matrix. It follows that a  $P_0^{(-)}$  matrix plus a negative diagonal matrix is a  $P^{(-)}$  matrix. Thus  $J$  is a  $P^{(-)}$  matrix.  $\square$

Combined with the result of Gale and Nikaido [10], this can be stated as the following corollary:

**COROLLARY 3.5.** *If the reactions in a CFSTR are NAC, and the stoichiometric matrix  $S$  is SSD, then the system does not admit multiple equilibria.*

This result is independent of the nature of the reactions (mass action, Michaelis-Menten, etc).

For CFSTR systems the result presented in Theorem 3.2 can be strengthened. If the stoichiometric matrix of true reactions in a CFSTR system is not SSD, and hence the Jacobian can fail to be a  $P^{(-)}$  matrix, then it can in fact be singular.

**THEOREM 3.6.** *Assume all the reactions in a CFSTR are NAC, and that the stoichiometric matrix of true reactions,  $S$ , is not SSD. Then there is some choice of entries in  $V$  for which  $\det(J)$  has sign  $(-1)^{n+1}$  (i.e. the “wrong” sign).*

*Proof.* The result follows as long as there is a term of the wrong sign in the expansion of the determinant, and this term can be made to dominate all other terms in the expansion.

As in the proof of Theorem 3.2, when  $S$  is not SSD, this implies the existence of sets  $\alpha_0 \subset \{1, \dots, n\}$ ,  $\gamma_0 \subset \{1, \dots, m\}$  with  $|\alpha_0| = |\gamma_0|$  such that  $V[\gamma_0|\alpha_0]$  contains a term  $t$  such that  $S[\alpha_0|\gamma_0]t$  has sign  $(-1)^{|\alpha_0|+1}$ .

Let  $S_f$  and  $V_f$  be defined as in the proof of Theorem 3.4. The structure of  $S_f$  and  $V_f$  means that there is a term in  $\det(S_f V_f)$  of the form  $(-q)^{n-|\alpha_0|} S[\alpha_0|\gamma_0]t$ , which is clearly of sign  $(-1)^{n+1}$ . As the determinant of any submatrix of  $V_f$  is a homogeneous polynomial in the entries of  $V_f$ , and no entry from  $V$  can occur more than once in any term, setting all entries in  $V$  other than those which occur in  $t$  to zero ensures that

$$\det(S_f V_f) = (-q)^{n-|\alpha_0|} S[\alpha_0|\gamma_0]t + \text{higher order terms in } q$$

Choosing any fixed values for entries in  $t$ , then for small enough  $q$ , the lowest order term  $(-q)^{n-|\alpha_0|} S[\alpha_0|\gamma_0]t$  is the dominant term in this expression, and hence  $\det(S_f V_f)$  has sign  $(-1)^{n+1}$ . As in the proof of Theorem 3.2, by continuity, the argument remains true for small non-zero entries in  $V_f$ .  $\square$

This last theorem is more important than it may at first seem. It implies that if  $S$  is not SSD then the Jacobian can be made singular by choosing entries in  $V$  appropriately. Thus finding that a particular reaction system has stoichiometric matrix which is SSD is a *necessary* condition to ensure that under arbitrary choice of dynamics the Jacobian of the CFSTR system can never be singular.

The astute reader will have noticed that combination of the previous theorems implies that for a CFSTR system nonsingularity of the Jacobian (for all entries in  $V$ ) is equivalent to injectivity of the system. This implies that when checking if a system is necessarily injective, rather than checking whether  $S$  is SSD one could instead check whether all  $n \times n$  submatrices of  $S_f$  are either SNS or singular. Although at first glance

the second strategy appears easier, the two problems are computationally equivalent, as computing the determinants of all  $n \times n$  submatrices of  $S_f$  requires computation of the determinants of all square submatrices of  $S$ .

**4.  $P^{(-)}$  matrices and mass action systems.** In this section we present some results on mass action systems. It is possible to prove stronger results about mass action systems than arbitrary reaction systems because the matrix  $V$  has additional structure beyond its sign structure. Our concern now is with the question of when a reaction system, as a result of its structure combined with the assumption of mass action dynamics, generates a  $P_0^{(-)}$  matrix Jacobian (or in the case of CFSTR systems, a  $P^{(-)}$  matrix Jacobian). Of course, if a substrate never occurs on both sides of any reaction, then the mass action form guarantees that all reactions are NAC, and so if the stoichiometric matrix  $S$  is SSD, this will ensure a  $P_0^{(-)}$  Jacobian. We show however that in the case of mass action systems it is possible to weaken the condition that  $S$  must be SSD and still get a  $P_0^{(-)}$  Jacobian.

It is important at the outset to highlight the close relationship between results in this section and results in [5]. The techniques for confirming whether a reaction system is injective given in [5] are more general than ours in that they apply to autocatalytic reactions as well. We are unable to make claims about injectivity of autocatalytic reactions using our techniques because the stoichiometric matrix “loses information” about reactions which have the same substrate occurring on both sides of the equation – it only encodes net production or loss of a substrate in a reaction, rather than absolute quantities on each side of a reaction. There is some overlap in our methods of proof although there are also important differences. We will return to this theme at the end of the section.

To formulate the results to follow we need to note that any mass action system can be written as a system of irreversible reactions by considering any reversible reaction as two irreversible reactions. From Lemma A.2 in Appendix A, rewriting the system in this way does not affect whether the stoichiometric matrix is SSD.

We now define a property of stoichiometric matrices weaker than the property of being SSD. Given a matrix  $S$ , define  $S_-$  to be the matrix  $S$  with all positive entries replaced with zeroes. Let a constant matrix  $S$  be **weakly sign determined** (WSD) if every square submatrix  $\tilde{S}$  of  $S$  satisfies  $\det(\tilde{S})\det(\tilde{S}_-) \geq 0$ . In Lemma A.3 of Appendix A it is shown that every SSD matrix is WSD. The two are not equivalent however – for example the matrix

$$\tilde{S} = \begin{bmatrix} 1 & -1 \\ -2 & 1 \end{bmatrix}$$

is neither SNS nor singular, but does satisfy  $\det(\tilde{S})\det(\tilde{S}_-) \geq 0$ . Results in Appendix A also show that the choice of how to order the set of substrates or reactions does not affect whether the stoichiometric matrix is WSD or not. However as we shall see later, the choice to represent one reversible reaction as two irreversible ones *can* affect whether the stoichiometric matrix is WSD or not.

We can now restate Theorem 3.1 for mass action systems.

**THEOREM 4.1.** *Consider the stoichiometric matrix  $S$  of a NAC mass action reaction system written as a system of irreversible reactions. If  $S$  is WSD, then the Jacobian  $J$  is a  $P_0^{(-)}$  matrix.*

*Proof.* The reaction rate for the  $i$ th reaction takes the form

$$v_i = k_i \prod_{j \in \nu_i} x_j^{-S_{ji}}$$

where  $k_i$  is the rate constant for the  $i$ th reaction and  $\nu_i$  is the set of indices of the reactants on the left hand side of the  $i$ th reaction. So the entries in  $V$  take the form:

$$V_{ij} = \frac{\partial v_i}{\partial x_j} = \begin{cases} \frac{-S_{ji}}{x_j} v_i & (j \in \nu_i) \\ 0 & (j \notin \nu_i) \end{cases}$$

As above, define  $S_-$  to be the matrix  $S$  with all positive entries replaced with zeroes. Further, let  $D_x$  be the  $n \times n$  positive diagonal matrix with entries  $\frac{1}{x_j}$  on the diagonal (defined when  $x_j > 0$  for all  $j$ ). Finally, let  $D_v$  be the  $m \times m$  positive diagonal matrix with entries  $v_i$  on the diagonal. With this notation the matrix  $V$  can be written

$$V = -D_v S_-^T D_x$$

(again formally defined only when all  $x_j > 0$ , although of course  $V$  exists in the limit as well). Now consider an arbitrary minor of  $V$ ,  $V[\gamma|\alpha]$  with  $\alpha \subset \{1, \dots, n\}$  and  $\gamma \subset \{1, \dots, m\}$ , and  $|\alpha| = |\gamma|$ . Application of the Cauchy-Binet formula combined with the fact that only principal minors of a diagonal matrix are nonzero gives

$$V[\gamma|\alpha] = (-1)^{|\alpha|} D_v[\gamma] S_-^T[\gamma|\alpha] D_x[\alpha]$$

So a principal minor of the Jacobian takes the form:

$$\begin{aligned} J[\alpha] &= (SV)[\alpha] = \sum_{\substack{\gamma \subset \{1, \dots, m\} \\ |\gamma| = |\alpha|}} S[\alpha|\gamma] V[\gamma|\alpha] \\ &= (-1)^{|\alpha|} \sum_{\substack{\gamma \subset \{1, \dots, m\} \\ |\gamma| = |\alpha|}} S[\alpha|\gamma] D_v[\gamma] S_-^T[\gamma|\alpha] D_x[\alpha] \\ &= (-1)^{|\alpha|} D_x[\alpha] \sum_{\substack{\gamma \subset \{1, \dots, m\} \\ |\gamma| = |\alpha|}} S[\alpha|\gamma] S_-[\alpha|\gamma] D_v[\gamma] \end{aligned}$$

Since  $D_x$  and  $D_v$  are positive diagonal matrices,  $D_x[\alpha]$  and  $D_v[\gamma]$  are positive. So  $J[\alpha]$  has sign  $(-1)^{|\alpha|}$  or is zero provided that every  $S[\alpha|\gamma]$  and  $S_-[\alpha|\gamma]$  have the same sign (or one of them is zero).

The argument presented above shows that if  $S$  is WSD then the Jacobian of a mass action system is a  $P_0^{(-)}$  matrix in the interior of the positive orthant. However the set of  $P_0^{(-)}$  matrices is closed, and since the Jacobian depends continuously on the values of  $x_i$ , it must be  $P_0^{(-)}$  everywhere in the closed positive orthant.  $\square$

The following corollary is immediate:

**COROLLARY 4.2.** *Assume all the reactions in a CFSTR are NAC mass action reactions. If the stoichiometric matrix  $S$  of the system written as a set of irreversible reactions is WSD then the Jacobian of the system is a  $P^{(-)}$  matrix.*

*Proof.* The proof is identical to that of Theorem 3.4: A  $P_0^{(-)}$  matrix plus a negative diagonal matrix is a  $P^{(-)}$  matrix.  $\square$

There is a kind of converse to Theorem 4.1 showing that the condition of being WSD is *necessary* to guarantee that the Jacobian of a mass action system will be a  $P_0^{(-)}$  matrix.

**THEOREM 4.3.** *Assume that the stoichiometric matrix  $S$  of a NAC mass action system written as a set of irreversible reactions is not WSD. Then there is some choice of rate constants  $k_i$  for which  $SV$  is not a  $P_0^{(-)}$  matrix.*

*Proof.* If  $S$  is not WSD, then  $S[\alpha_0|\gamma_0]S_-[\alpha_0|\gamma_0] < 0$  for some  $\alpha_0 \subset \{1, \dots, n\}$ ,  $\gamma_0 \subset \{1, \dots, m\}$  with  $|\alpha_0| = |\gamma_0|$ . We have from above:

$$J[\alpha_0] = (-1)^{|\alpha_0|} D_x[\alpha_0] \sum_{\substack{\gamma \subset \{1, \dots, m\} \\ |\gamma| = |\alpha_0|}} S[\alpha_0|\gamma] S_-[\alpha_0|\gamma] D_v[\gamma]$$

Since  $D_v[\gamma] = \prod_{j \in \gamma} v_j$ , choosing  $k_j = 0$  for all  $j \notin \gamma_0$ , and  $k_j \neq 0$  for all  $j \in \gamma_0$ , sets all  $D_v[\gamma] = 0$  for  $\gamma \not\subset \gamma_0$ . So with this choice

$$J[\alpha_0] = (-1)^{|\alpha_0|} D_x[\alpha_0] S[\alpha_0|\gamma_0] S_-[\alpha_0|\gamma_0] D_v[\gamma_0]$$

which has sign  $(-1)^{|\alpha_0|+1}$  everywhere in the interior of the positive orthant. By continuity,  $J[\alpha_0]$  continues to have sign  $(-1)^{|\alpha_0|+1}$  in some region of the positive orthant when  $k_j, j \notin \gamma_0$ , are small but nonzero.  $\square$

For mass action systems, the condition of being WSD is thus *necessary* to guarantee that the Jacobian will be a  $P_0^{(-)}$  matrix. In fact in the case of CFSTR mass action systems there is an analogue of the general result in Theorem 3.6: the property of  $S$  being WSD is necessary to guarantee that the Jacobian will be nonsingular.

**THEOREM 4.4.** *Assume that the stoichiometric matrix  $S$  of the true reactions in a NAC mass action CFSTR system written as a set of irreversible reactions is not WSD. Then there is some choice of flow rate  $q$ , rate constants  $k_i$ , and concentrations  $x_i$  for which  $\det(J)$  has sign  $(-1)^{n+1}$  (i.e. the “wrong” sign).*

*Proof.* The proof is a little harder than the equivalent proof for general systems, but again, the result follows as long as there is a term of the wrong sign in the expansion of the determinant, and this term can be made to dominate all other terms in the expansion.

Since  $S$  is not WSD,  $S[\alpha_0|\gamma_0]S_-[\alpha_0|\gamma_0] < 0$  for some sets  $\alpha_0 \subset \{1, \dots, n\}$ ,  $\gamma_0 \subset \{1, \dots, m\}$  with  $|\alpha_0| = |\gamma_0|$ .

The Jacobian  $J = SV - qI$  and the determinant of the Jacobian is  $\det(SV - qI)$ . Expanding this we get

$$\begin{aligned} \det(SV - qI) &= \sum_{j=0}^n (-1)^j q^j \sum_{\substack{\alpha \subset \{1, \dots, n\} \\ |\alpha| = n-j}} SV[\alpha] \\ &= \sum_{j=0}^n (-1)^j q^j \sum_{\substack{\alpha \subset \{1, \dots, n\} \\ |\alpha| = n-j}} (-1)^{n-j} D_x[\alpha] \sum_{\substack{\gamma \subset \{1, \dots, m\} \\ |\gamma| = |\alpha|}} S[\alpha|\gamma] S_-[\alpha|\gamma] D_v[\gamma] \\ &= (-1)^n \sum_{j=0}^n q^j \sum_{\substack{\alpha \subset \{1, \dots, n\} \\ |\alpha| = n-j}} D_x[\alpha] \sum_{\substack{\gamma \subset \{1, \dots, m\} \\ |\gamma| = |\alpha|}} S[\alpha|\gamma] S_-[\alpha|\gamma] D_v[\gamma] \end{aligned}$$

Setting all  $k_i \notin \gamma_0$  equal to zero we get

$$\det(SV - qI) = (-1)^n q^{n-|\gamma_0|} D_v[\gamma_0] \sum_{\substack{\alpha \subset \{1, \dots, n\} \\ |\alpha| = |\gamma_0|}} D_x[\alpha] S[\alpha|\gamma_0] S_-[\alpha|\gamma_0] \\ + \text{higher order terms in } q$$

We know that  $S[\alpha_0|\gamma_0] S_-[\alpha_0|\gamma_0] < 0$ . Since  $D_x[\alpha] = \prod_{i \in \alpha} x_i^{-1}$ , by fixing values of  $x_i$  for  $i \in \alpha_0$  and increasing the values of  $x_i$  for  $i \notin \alpha_0$  we can make  $D_x[\alpha_0]$  much larger than  $D_x[\alpha]$  for any  $\alpha \neq \alpha_0$  in the sum above, thus ensuring that the term

$$D_v[\gamma_0] D_x[\alpha_0] S[\alpha_0|\gamma_0] S_-[\alpha_0|\gamma_0]$$

is the dominant term in the coefficient of  $q^{n-|\gamma_0|}$  and thus that this coefficient has sign  $(-1)^{n+1}$ . (Note that increasing the values of  $x_i \notin \alpha_0$  affects, but can never decrease, the size of  $D_x[\alpha_0] D_v[\gamma_0]$ .)

Once we have ensured that the coefficient of  $q^{n-|\gamma_0|}$  has sign  $(-1)^{n+1}$ , we can choose  $q$  small so that the term of order  $q^{n-|\gamma_0|}$  is the dominant term in  $\det(SV - qI)$ . Thus for small  $q$ , small  $x_i \in \alpha_0$  (and all other  $x_i$  sufficiently large), large  $k_i \in \gamma_0$  (and all other  $k_i$  sufficiently small) we can ensure that  $\det(SV - qI)$  has sign  $(-1)^{n+1}$ .  $\square$

This final result shows that if  $S$  is not WSD, then for some choices of rate constants and flow rate the Jacobian of a CFSTR system will be singular. Thus the property of  $S$  being WSD is both sufficient and *necessary* to ensure that the Jacobian of a NAC mass action CFSTR system is always nonsingular. It is also sufficient and *necessary* to ensure that the Jacobian is always a  $P^{(-)}$  matrix and hence that the system is injective. Together these facts imply that nonsingularity of the Jacobian of a NAC mass action CFSTR system is equivalent to injectivity for these systems. This theorem overlaps with Theorem 3.3 in [5]: Both theorems rely on the fact that for the polynomials which define the determinants in CFSTR systems positivity of the numerical coefficients is necessary to ensure positivity of the polynomial.

There are further close relationships between the theorems here and those in [5]. In Theorem 3.1 of [5] it is proved directly that mass action systems are injective if and only if their Jacobians are nonsingular for all positive values of the rate constants and concentrations. As just related, we come to the same conclusion for NAC systems via a different route: We have proved that the condition that  $S$  is WSD is equivalent both to injectivity and to nonsingularity of the Jacobian in the CFSTR case, and thus that these two are themselves equivalent. This in turn implies that the condition that the stoichiometric matrix of true reactions must be WSD and the requirement that the quantity in equation (3.4) of [5] must be positive are equivalent for NAC mass action systems embedded in a CFSTR.

One apparent difference between the results here and those in [5] lies in the fact that in Theorem 3.2 of [5], only determinants of  $n \times n$  submatrices of the *full* stoichiometric matrix are needed, whereas when checking the WSD condition we have to check all square submatrices of the stoichiometric matrix. However this difference is only apparent, and the remark that we made about general systems applies again here: Checking whether  $S$  is WSD is computationally equivalent to checking whether all  $n \times n$  submatrices  $T$  of  $S_f = [S | -I_n]$  satisfy  $\det(\tilde{T}) \det(\tilde{T}_-) \geq 0$ .

**5. Examples.** We present some examples to illustrate the theoretical points in the previous sections.

**5.1. Examples from [5].** The phenomenon of  $S$  being SSD is more common than it might at first seem. We first examined the reaction system (1.1) in [5] and examples (i) to (viii) presented in Table 1.1 of this reference. Of these, examples (vi), (vii) and (viii) have reactants on both sides of the reactions, and are discussed below in Section 5.4. Our analysis of the other examples is presented in Table 5.1. In all cases, we found that whether or not the system had the capacity for multiple equilibria corresponded precisely to whether or not the stoichiometric matrix was SSD. We can thus state for the three systems in which multiple equilibria were ruled out – system (1.1), and systems (ii) and (iv) in Table 1.1 – that this remains true if we violate the mass action assumption.

It is no surprise that the NAC systems which were proved to be injective in [5] proved to be WSD since, as shown in the previous section, the stoichiometric matrix being WSD is necessary for injectivity of the Jacobian for all values of the rate constants. What is surprising is that all of these examples turned out also to be SSD, and thus that the conclusions about these systems in [5] turn out to be more generally true.

	reaction system	SSD	WSD
i)	$A + B \rightleftharpoons P$ $B + C \rightleftharpoons Q$ $C \rightleftharpoons 2A$	not SSD	not WSD
ii)	$A + B \rightleftharpoons P$ $B + C \rightleftharpoons Q$ $C + D \rightleftharpoons R$ $D \rightleftharpoons 2A$	SSD	WSD
iii)	$A + B \rightleftharpoons P$ $B + C \rightleftharpoons Q$ $C + D \rightleftharpoons R$ $D + E \rightleftharpoons S$ $E \rightleftharpoons 2A$	not SSD	not WSD
iv)	$A + B \rightleftharpoons P$ $B + C \rightleftharpoons Q$ $C \rightleftharpoons A$	SSD	WSD
v)	$A + B \rightleftharpoons F$ $A + C \rightleftharpoons G$ $C + D \rightleftharpoons B$ $C + E \rightleftharpoons D$	not SSD	not WSD
Ex. 1.1	$A + B \rightleftharpoons C$ $X \rightleftharpoons 2A + D$ $2A + D \rightleftharpoons Y$ $D \rightleftharpoons C + W$ $B + D \rightleftharpoons Z$	SSD	WSD

TABLE 5.1

*Behaviour of some reaction systems presented in [5]. In all the examples where the systems are WSD, the systems are also in fact SSD, and thus multiple equilibria are ruled out in a CFSTR under arbitrary dynamics.*

**5.2. Systems which are WSD but not SSD.** Although the examples taken from [5] and presented in Table 5.1 are all either both SSD and WSD or neither, it is possible to construct examples of systems which are WSD but not SSD. Consider the reaction system



which has stoichiometric matrix, in reversible and irreversible forms

$$S_r = \begin{bmatrix} -1 & -2 \\ -1 & -1 \\ 1 & 0 \\ 0 & 1 \end{bmatrix} \quad S_{ir} = \begin{bmatrix} -1 & 1 & -2 & 2 \\ -1 & 1 & -1 & 1 \\ 1 & -1 & 0 & 0 \\ 0 & 0 & 1 & -1 \end{bmatrix}$$

It is quick to check that  $S_r$  (and hence  $S_{ir}$ ) are not SSD. On the other hand  $S_{ir}$  is WSD. Thus if these reactions are embedded in a CFSTR, multiple equilibria can be ruled out as long as the dynamics are mass action dynamics, but not in the general case.

**5.3. The reaction system as a reversible/irreversible system.** To illustrate that it is essential to consider a system as a set of irreversible reactions when checking whether a stoichiometric matrix is WSD or not, consider the following reaction system:



which has stoichiometric matrix, in reversible and irreversible forms

$$S_r = \begin{bmatrix} -2 & -1 \\ 1 & 1 \\ 1 & 0 \end{bmatrix} \quad S_{ir} = \begin{bmatrix} -2 & 2 & -1 & 1 \\ 1 & -1 & 1 & -1 \\ 1 & -1 & 0 & 0 \end{bmatrix}$$

Here  $S_r$  is WSD, but  $S_{ir}$  is not. Thus examining  $S_r$  alone could give rise to the wrong conclusion that multiple equilibria can be ruled out in the mass action case.

This example also illustrates the importance of reversibility in the mass-action case. Consider the above system with one reaction now irreversible:

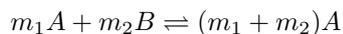


This has stoichiometric matrix, in irreversible form:

$$S_{ir} = \begin{bmatrix} -2 & 2 & 1 \\ 1 & -1 & -1 \\ 1 & -1 & 0 \end{bmatrix}$$

which is in fact WSD. Thus with mass action dynamics this system does not admit multiple equilibria when embedded in a CFSTR. It is perhaps surprising that if the reaction  $B \rightarrow A$  were replaced with  $A \rightarrow B$  then the system would no longer be WSD and the conclusion would no longer hold. Instead, for certain choices of the rate constants, the system would cease to be injective, and multiple equilibria, while not guaranteed, can no longer be ruled out by this method.

**5.4. Autocatalytic reactions.** Consider the reactions in [5] of the form



for some positive integers  $m_1$  and  $m_2$ . Recasting these as



and assuming NAC dynamics gives rise to stoichiometric matrices, in reversible and irreversible forms

$$S_r = \begin{bmatrix} -m_1 & (m_1 + m_2) \\ -m_2 & 0 \\ 1 & -1 \end{bmatrix} \quad S_{ir} = \begin{bmatrix} -m_1 & m_1 & (m_1 + m_2) & -(m_1 + m_2) \\ -m_2 & m_2 & 0 & 0 \\ 1 & -1 & -1 & 1 \end{bmatrix}$$

Barring the trivial possibilities that  $m_1 = 0$  or  $m_2 = 0$ ,  $S_r$  is never SSD and  $S_{ir}$  is never WSD. Thus multiple equilibria cannot be ruled out in general or for mass action systems. However for mass action dynamics in the cases  $m_1 = 1, m_2 = 1$  and  $m_1 = 1, m_2 = 2$ , it is known that multiple equilibria cannot exist [5], illustrating that singularity of the Jacobian is not sufficient to guarantee multiple equilibria. This is because although a function in some class may fail to be injective, the class may not allow this failure to occur near its zeros.

In fact it is easy to show that when a reactant occurs on both sides of a reaction with different stoichiometries, and we rewrite the system as two NAC reactions with an intermediate complex, the system cannot be SSD or WSD. Consider the reaction system



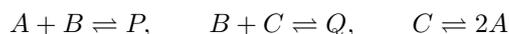
which might result from such a rewriting. Assume for definiteness that  $m > n$ . Then the irreversible stoichiometric matrix  $S_{ir}$  has a  $2 \times 2$  submatrix of the form:

$$T = \begin{bmatrix} -n & m \\ 1 & -1 \end{bmatrix}$$

which is clearly not SNS, not singular, and does not satisfy  $\det(T)\det(T_-) \geq 0$  either.

**5.5. Computational considerations.** Although it is easy to write down algorithms to check whether a given matrix is SSD or WSD, the actual computation involves checking a large number of submatrices, and can be lengthy if the reaction network is large. Since large stoichiometric matrices are in general highly sparse, considerable speed-up can be achieved by using algorithms to identify submatrices which have (identically) zero determinant without actually attempting to compute the determinant. Similarly, intelligent algorithms should avoid recomputation of the determinants of matrices when they occur as submatrices in larger matrices.

Another technique which can speed up the classification of a matrix as SSD or WSD relies on the fact that it is possible to ignore all substrates which occur in only one reaction as shown in Lemmas A.4-A.6 in Appendix A. This greatly shortens the calculations in many real examples. Consider Example (i) in Table 5.1:



which has stoichiometric matrix, in reversible and irreversible forms

$$S_r = \begin{bmatrix} -1 & 0 & 2 \\ -1 & -1 & 0 \\ 0 & -1 & -1 \\ 1 & 0 & 0 \\ 0 & 1 & 0 \end{bmatrix} \quad S_{ir} = \begin{bmatrix} -1 & 1 & 0 & 0 & 2 & -2 \\ -1 & 1 & -1 & 1 & 0 & 0 \\ 0 & 0 & -1 & 1 & -1 & 1 \\ 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 & 0 \end{bmatrix}$$

Since  $P$  and  $Q$  each only occur in a single reaction, when checking whether the system is SSD and WSD respectively it suffices to check the reduced matrices

$$\tilde{S}_r = \begin{bmatrix} -1 & 0 & 2 \\ -1 & -1 & 0 \\ 0 & -1 & -1 \end{bmatrix} \quad \tilde{S}_{ir} = \begin{bmatrix} -1 & 1 & 0 & 0 & 2 & -2 \\ -1 & 1 & -1 & 1 & 0 & 0 \\ 0 & 0 & -1 & 1 & -1 & 1 \end{bmatrix}$$

which considerably reduces the computational effort.

**6. Rate dependent negative feedback processes.** Having seen that NAC reaction systems often give rise to Jacobians which are  $P_0^{(-)}$  or  $P^{(-)}$  matrices, and how this property is deeply associated with *reaction structure* rather than reaction details, we now examine the process of adding a rate dependent negative feedback process to a given system – i.e. adding a scalar quantity  $\psi$  (perhaps the concentration of another reactant) which inhibits or activates reactions according to whether it is produced or used up in them. We also allow  $\psi$  to be subject to an outflow/degradation process.

The full system becomes

$$\dot{x} = Sv(x, \psi), \quad \dot{\psi} = C(v(x, \psi)) - L(\psi) \quad (6.1)$$

The function  $C(v)$  represents the reaction-rate dependent creation of  $\psi$  while  $L(\psi)$  represents its level-dependent outflow or degradation.

We define the following quantities:

1.  $F \equiv \frac{\partial v}{\partial \psi}$ . This  $m$  vector describes the dependence of the reaction rates on  $\psi$
2.  $P \equiv \left(\frac{\partial C}{\partial v}\right)^T$ . This  $m$  vector describes the way that the production of  $\psi$  depends on the reaction rates
3.  $u \equiv \frac{\partial L}{\partial \psi}$ . This scalar describes the rate of decay of  $\psi$ .

The most general mathematical meaning of the negative feedback assumption is that the vectors  $F$  and  $P$  lie in opposite cones generated by some set of  $m$  orthogonal vectors. Thus for some orthogonal transformation  $U$ ,

$$P \in K \equiv U(\mathbb{R}_+^m) \quad \text{and} \quad F \in -K$$

The case generally encountered in examples is where  $K$  is a particular orthant so that  $U$  is a so-called signature matrix (a diagonal matrix with diagonal entries  $\pm 1$ ) and we know the signs of the elements of  $P$  and  $F$ , but not their values. In fact we will initially assume that  $U = I$ , the identity matrix, i.e.  $P \in \mathbb{R}_+^m$  and  $F \in \mathbb{R}_-^m$ , showing later how the results can be extended to the general case.

The Jacobian of (6.1) is now the key object of interest. It can be written in block form:

$$J = \begin{bmatrix} SV & SF \\ P^T V & P^T F - u \end{bmatrix} \quad (6.2)$$

We will prove all the results in this section by examining matrices (and submatrices) of the form  $J$  above. In order to discuss the negative feedback assumption we adopt the following standard notation ([3] for example). Given a vector  $y \in \mathbb{R}^n$

- $y \geq 0$  will mean that  $y_i \geq 0$  for all  $i$
- $y > 0$  will mean that  $y \geq 0$  and  $y \neq 0$
- $y \gg 0$  will mean that  $y_i > 0$  for all  $i$
- $y \leq 0$ ,  $y < 0$  and  $y \ll 0$  will have analogous meanings.

With this notation, the negative feedback assumption can be rephrased as  $F < 0$  and  $P > 0$ . We will assume that  $u > 0$  and make claims about when  $J$  is a  $P^{(-)}$  matrix – the extension to  $u = 0$  and the  $P_0^{(-)}$  case will be automatic, using the continuous dependence of determinants on the entries in a matrix.

Having discussed these preliminaries, we now ask the following question: Assuming that  $SV$  is a  $P^{(-)}$  matrix, under what conditions will the Jacobian  $J$  in (6.2) remain a  $P^{(-)}$  matrix for all values of  $F < 0$ ,  $P > 0$  and  $u > 0$ ? The complete answer to this question is contained in Theorems 6.2 and 6.3.

In what is to follow, we will make use of the following formula for the determinant of a matrix.

LEMMA 6.1. *Let  $A$  be any matrix written in block form as follows:*

$$A = \begin{bmatrix} A_{11} & A_{12} \\ A_{21} & A_{22} \end{bmatrix}$$

where  $A_{11}$  and  $A_{22}$  are square matrices. Assuming  $A_{11}$  is nonsingular, then:

$$\det(A) = \det(A_{22} - A_{21}A_{11}^{-1}A_{12})\det(A_{11})$$

*Proof.* See for example [16], p46.  $\square$

Note that if  $A_{22}$  is a scalar, the equation becomes

$$\det(A) = (A_{22} - A_{21}A_{11}^{-1}A_{12})\det(A_{11})$$

We now state the basic theorem about the determinant of Jacobians of the form in (6.2) from which results on  $P^{(-)}$  properties will follow easily.

THEOREM 6.2. *Consider a matrix  $J$  of the form in (6.2). Define  $\mathcal{S} = \ker(S)$  and  $\mathcal{V} = \ker(V^T)$ . Assume that  $\det(SV)$  has sign  $(-1)^n$ . Define  $Z \equiv V(SV)^{-1}S$ . Then the following statements are equivalent:*

- (A) *Given any vector  $w_1 \in \mathcal{S}$  satisfying  $w_1 \not\geq 0$ , we can find a vector  $w_2 \in \mathcal{V}$  satisfying  $w_2 > 0$  such that  $\langle w_1, w_2 \rangle < 0$ .*
- (B)  *$\det(J)$  has sign  $(-1)^{n+1}$  for any choice of  $F < 0$ ,  $P > 0$  and  $u > 0$ .*
- (C)  *$I - Z$  is a nonnegative matrix.*

Before we begin proof of the theorem we discuss a couple of the assumptions. The assumption that  $\det(SV)$  is always of sign  $(-1)^n$  implies that  $\ker(V)$  and  $\ker(S^T)$  consist only of 0. The condition that  $\ker(S^T)$  consists only of 0 in turn means that there are no conserved quantities in the system [8], certainly true in the CFSTR case. In any case, where there are conserved quantities, the system can generally be redefined with some variables being eliminated to remove these.

A situation in which the theorem is trivially satisfied is when  $S$  and hence  $V$  are square matrices – i.e. there are the same number of substrates and reactions. Then

the condition that  $SV$  is nonsingular implies that  $S$  and  $V$  are both nonsingular and hence both  $\mathcal{S}$  and  $\mathcal{V}$  consist only of zero, and there are no vectors  $w_1 \in \mathcal{S}$  satisfying  $w_1 \not\geq 0$ . In this case the matrix  $Z$  is the identity. As mentioned earlier, if  $S$  is a nonsingular square matrix, then any equilibria correspond to all reaction rates being zero.

During the proof, we will see that Condition (A) of the theorem has the following geometric interpretation: It means that the projection of any nonnegative vector  $y > 0$  along  $\mathcal{V}^T$  onto  $\mathcal{S}$  is nonnegative. We remark that there is an important special case where Condition (A) is immediately satisfied – this is when  $\mathcal{S}$  is one dimensional, lying entirely in the nonnegative and nonpositive orthants, and  $\mathcal{V}$  contains some strictly positive vectors. Then given  $w_1 \in \mathcal{S}$ ,  $w_1 \not\geq 0 \Rightarrow w_1 < 0$ , and given any  $w_2 \in \mathcal{V}$  satisfying  $w_2 \gg 0$  we have  $\langle w_1, w_2 \rangle < 0$ . In general, however, where  $\mathcal{S}$  can intersect other orthants, the existence of the vector  $w_2$  will depend on the structures of  $\mathcal{S}$  and  $\mathcal{V}$ .

*Proof.* Proof of Theorem 6.2

We show that both (A) and (B) are equivalent to (C), starting with (B)  $\Leftrightarrow$  (C). Using Lemma 6.1 we get that

$$\begin{aligned} \det(J) &= (P^T F - u - P^T V(SV)^{-1} S F) \det(SV) \\ &= (P^T (I - Z) F - u) \det(SV) \end{aligned}$$

where  $I$  is the  $m \times m$  unit matrix. Since  $\det(SV)$  has sign  $(-1)^n$ , this means immediately that (6.2) will have determinant of sign  $(-1)^{n+1}$  as long as  $P^T (I - Z) F - u < 0$ . This is true for all  $u > 0$  if and only if  $P^T (I - Z) F \leq 0$ . This in turn is true for all  $P > 0$ , and  $F < 0$  if and only if  $(I - Z)$  is a nonnegative matrix (i.e. it leaves the nonnegative orthant invariant). Otherwise we can choose  $F$  and  $P$  appropriately so that  $P^T (I - Z) F > 0$ . Thus (B)  $\Leftrightarrow$  (C).

We now show that (A)  $\Rightarrow$  (C). It is easy to see that  $Z^2 = Z$  – i.e.  $Z$  is a projection. As  $SV$  is nonsingular,  $\ker(Z) = \ker(S) = \mathcal{S}$  and  $\ker(Z^T) = \ker(V^T) = \mathcal{V}$ . Thus  $Z$  acts as a projection along  $\mathcal{S}$  onto  $\mathcal{V}^\perp$  and  $I - Z$  projects along  $\mathcal{V}^\perp$  onto  $\mathcal{S}$ .

Consider an arbitrary vector  $y > 0$ . Write  $y = y_1 + y_2$  where  $y_1 \equiv (I - Z)y \in \mathcal{S}$  and  $y_2 \equiv Zy \in \mathcal{V}^\perp$ . Now if  $y_1 \not\geq 0$ , then by assumption we can choose a vector  $z \in \mathcal{V}$  satisfying  $z > 0$  and  $\langle z, y_1 \rangle < 0$ . But then  $\langle z, y \rangle = \langle z, y_1 \rangle < 0$  contradicting the fact that  $z > 0$  and  $y > 0$ . So  $y_1 \geq 0$ . Thus  $(I - Z)$  leaves the nonnegative orthant invariant, and is a nonnegative matrix. Thus (A)  $\Rightarrow$  (C).

Finally, (C)  $\Rightarrow$  (A): If  $(I - Z)$  is a nonnegative matrix we show that given any  $y_1 \in \mathcal{S}$  satisfying  $y_1 \not\geq 0$ , there is a  $z \in \mathcal{V}$  satisfying  $z > 0$  such that  $\langle z, y_1 \rangle < 0$ . Note that if  $y_1 \not\geq 0$  then there is some vector  $r \gg 0$  such that  $\langle r, y_1 \rangle < 0$ . So

$$0 > \langle r, y_1 \rangle = \langle r, (I - Z)y_1 \rangle = \langle (I - Z^T)r, y_1 \rangle$$

Now note that  $(I - Z^T)r > 0$  because  $(I - Z^T) = (I - Z)^T$  is a nonnegative matrix and  $r \gg 0$ . Moreover  $(I - Z^T)r \in \mathcal{V}$  since  $(I - Z^T)$  is a projection along  $\mathcal{S}^\perp$  onto  $\mathcal{V}$ . So  $z \equiv (I - Z^T)r$  is a positive vector in  $\mathcal{V}$  which satisfies  $\langle z, y_1 \rangle < 0$ .  $\square$

Theorem 6.2 leads immediately to the following:

**THEOREM 6.3.**

Let  $J$  be a matrix of the form defined in (6.2). Let  $\alpha$  be some subset of  $\{1, \dots, n\}$ ,  $S^\alpha$  be the matrix  $S$  with rows belonging to  $\alpha$  deleted and  $V^\alpha$  the matrix  $V$  with columns belonging to  $\alpha$  deleted. Define  $\mathcal{S}^\alpha = \ker(S^\alpha)$  and  $\mathcal{V}^\alpha = \ker((V^\alpha)^T)$ .

Assume that  $SV$  is a  $P^{(-)}$  matrix. Then the following statements are equivalent:

- (A) For every  $\alpha \subset \{1, \dots, n\}$ , given any vector  $w_1 \in \mathcal{S}^\alpha$  satisfying  $w_1 \not\geq 0$ , we can find a vector  $w_2 \in \mathcal{V}^\alpha$  satisfying  $w_2 > 0$  such that  $\langle w_1, w_2 \rangle < 0$ .
- (B)  $J$  is a  $P^{(-)}$  matrix for any choice of  $F < 0$ ,  $P > 0$  and  $u > 0$ .

*Proof.* Since  $SV$  is a  $P^{(-)}$  matrix, to prove that  $J$  is a  $P^{(-)}$  matrix it suffices to treat all the principal submatrices of  $J$  obtained by deleting a set of rows/columns *not* including the final row and column. We show that for any  $\alpha \subset \{1, \dots, n\}$  the principal minor corresponding to the deletion of rows and columns from  $\alpha$  has sign  $(-1)^{n+1-|\alpha|}$ .

In the trivial case where  $\alpha = \{1, \dots, n\}$ , the principal submatrix corresponding to the removal of rows and columns from  $\alpha$  is simply the scalar  $P^T F - u$  which we know to be negative. In the case where  $\alpha = \emptyset$ , the principal submatrix is  $J$  itself. In general the principal submatrix corresponding to the removal of rows and columns from  $\alpha$  is:

$$J^\alpha \equiv \begin{bmatrix} S^\alpha V^\alpha & S^\alpha F \\ P^T V^\alpha & P^T F - u \end{bmatrix}$$

$S^\alpha V^\alpha$  is a principal submatrix of  $SV$  and since  $SV$  is a  $P^{(-)}$  matrix, its determinant has sign  $(-1)^{n-|\alpha|}$ .  $J^\alpha$  is of the form in (6.2), and to prove that  $\det(J^\alpha)$  has sign  $(-1)^{n+1-|\alpha|}$  it suffices by Theorem 6.2 that given any vector  $w_1 \in \mathcal{S}^\alpha$  satisfying  $w_1 \not\geq 0$ , we can find a vector  $w_2 \in \mathcal{V}^\alpha$  satisfying  $w_2 > 0$  such that  $\langle w_1, w_2 \rangle < 0$ .

The converse result follows because of the sufficiency of the condition set out in Theorem 6.2.  $\square$

Although the results above are about the  $P^{(-)}$  case, they extend to the  $P_0^{(-)}$  case:

**COROLLARY 6.4.** *Assume the conditions of Theorem 6.3 are fulfilled and hence that the matrix  $J$  in (6.2) is a  $P^{(-)}$  matrix for all values of  $F < 0$ ,  $P > 0$  and  $u > 0$ . Then  $J$  is a  $P_0^{(-)}$  matrix for all values of  $F \leq 0$ ,  $P \geq 0$  and  $u \geq 0$ .*

*Proof.* Given any particular  $\tilde{F} \leq 0$ ,  $\tilde{P} \geq 0$  and  $\tilde{u} \geq 0$  and  $J$  constructed using these, we can construct a sequence of  $P^{(-)}$  matrices  $\{J_i\}$  converging to  $J$  by choosing sequences  $\{F_i\} < 0$ ,  $\{P_i\} > 0$  and  $\{u_i\} > 0$  converging to  $\tilde{F}$ ,  $\tilde{P}$  and  $\tilde{u}$  respectively. Thus  $J$  lies in the closure of the  $P^{(-)}$  matrices and must be a  $P_0^{(-)}$  matrix.  $\square$

**6.1. Extension to the general case.** We now show briefly how the arguments in Theorems 6.2 and 6.3 extend to the general case where  $U$  is some orthogonal transformation,  $K = U(\mathbb{R}_+^m)$ ,  $P \in K$  and  $F \in -K$ . For arbitrary orthogonal  $U$ , the statement of Theorem 6.2 modifies to:

**THEOREM 6.5.** *Consider a matrix of the form  $J$  in (6.2) and let  $U$  be any  $m \times m$  orthogonal matrix. Define  $K = U(\mathbb{R}_+^m)$ ,  $\mathcal{S} = \ker(SU)$  and  $\mathcal{V} = \ker(V^T U)$ . Assume that  $\det(SV)$  has sign  $(-1)^n$ . Define  $Z \equiv V(SV)^{-1}S$ . Then the following statements are equivalent:*

- (A) Given any vector  $w_1 \in \mathcal{S}$  satisfying  $w_1 \not\geq 0$ , we can find a  $w_2 \in \mathcal{V}$  satisfying  $w_2 > 0$  such that  $\langle w_1, w_2 \rangle < 0$ .
- (B)  $\det(J)$  has sign  $(-1)^{n+1}$  for any choice of  $F \in -K$ ,  $P \in K$  and  $u > 0$ .
- (C)  $I - U^T Z U$  is a nonnegative matrix

*Proof.* The proof is identical to that of Theorem 6.2. In following through the steps the only things to note are that  $U^T P \in \mathbb{R}_+^m$  and  $U^T F \in \mathbb{R}_-^m$ . Further

$$\begin{aligned} P^T(I - Z)F &= P^T U U^T (I - Z) U U^T F \\ &= P^T U (I - U^T Z U) U^T F \end{aligned}$$

and  $U^T Z U$  is a projection, now projecting along  $\ker(SU)$  onto  $\ker(V^T U)$ .  $\square$

Similarly, Theorem 6.3 extends to

**THEOREM 6.6.**

Let  $J$  be a matrix of the form defined in (6.2) and  $U$  be any orthogonal matrix. Define  $K = U(\mathbb{R}_+^m)$ . Let  $\alpha$  be some subset of  $\{1, \dots, n\}$ ,  $S^\alpha$  be the matrix  $S$  with rows belonging to  $\alpha$  removed and  $V^\alpha$  the matrix  $V$  with columns belonging to  $\alpha$  removed. Define  $\mathcal{S}^\alpha = \ker(S^\alpha U)$  and  $\mathcal{V}^\alpha = \ker((V^\alpha)^T U)$ .

Assume that  $SV$  is a  $P^{(-)}$  matrix. Then the following statements are equivalent:

- (A) For every  $\alpha$ , given any vector  $w_1 \in \mathcal{S}^\alpha$  satisfying  $w_1 \not\geq 0$ , we can find a vector  $w_2 \in \mathcal{V}^\alpha$  satisfying  $w_2 > 0$  such that  $\langle w_1, w_2 \rangle < 0$ .
- (B)  $J$  is a  $P^{(-)}$  matrix for any choice of  $P \in K$ ,  $F \in -K$  and  $u > 0$ .

*Proof.* The proof follows directly from that of Theorem 6.5.  $\square$

**6.2. Two examples.** The way the above theorems can be used is seen in the two examples to follow. The first is a rather trivial example for illustrative purposes; the second is considerably harder and arises from a real biological system.

It is appropriate to mention that because for NAC reaction systems (see Section 3)  $S$  and  $V^T$  have opposite sign structures, there are certain natural relationships between  $\ker(S^\alpha)$  and  $\ker((V^T)^\alpha)$ . However this fact alone does not imply that Condition (A) of Theorem 6.2 is automatically fulfilled. Our first example illustrates this.

**Example 1.** Consider an open reaction system in which a single substrate  $x$  is involved in three processes, one of which produces a molecule of  $x$ , one of which produces two molecules of  $x$  and one of which degrades a molecule of  $x$ , so that  $S = [1, 2, -1]$ . Let  $V^T = [-a, -b, c]$  where  $a, b, c \geq 0$  and not all are equal to zero. Now  $SV = -(a + 2b + c) < 0$  so the Jacobian of the basic system is a negative scalar and hence a  $P^{(-)}$  matrix.  $\mathcal{S}$  is the plane in  $\mathbb{R}^3$  satisfying  $x_1 + 2x_2 - x_3 = 0$  while  $\mathcal{V}$  is the plane satisfying  $ax_1 + bx_2 - cx_3 = 0$ .

Now assume that there is a rate dependent feedback process such that the three reactions all create and are inhibited by some quantity. With  $P_i$  and  $F_i$  taking their usual meanings, we can check that the Jacobian of the system is

$$J = \begin{bmatrix} -(a + 2b + c) & F_1 + 2F_2 - F_3 \\ -aP_1 - bP_2 + cP_3 & P_1F_1 + P_2F_2 + P_3F_3 - u \end{bmatrix}$$

It is not immediately obvious by inspection that there are indeed choices of  $P_i \geq 0$  and  $F_i \leq 0$  for which  $J$  is not a  $P^{(-)}$  matrix, but an easy calculation gives

$$I - V(SV)^{-1}S = \frac{1}{(a + 2b + c)} \begin{bmatrix} 2b + c & -2a & a \\ -b & a + c & b \\ c & 2c & a + 2b \end{bmatrix}$$

which is clearly not a nonnegative matrix unless  $a$  and  $b$  are both zero. So, for small  $u > 0$  and some choices of  $P$  and  $F$ ,  $J$  is indeed not a  $P^{(-)}$  matrix, and can in fact be singular. Thus the  $P^{(-)}$  matrix property can be destroyed by a rate dependent negative feedback process for this NAC reaction system.

**Example 2.** In [1] a model of mitochondrial metabolism was presented consisting of a system of coupled redox reactions some of which interacted with the proton gradient across the mitochondrial membrane. Without this gradient the Jacobian of the system could be written as the product of a matrix

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

and a matrix  $V$ :

$$V = \begin{bmatrix} f_{11} & 0 & 0 & \cdots & 0 & 0 \\ -F_{21} & f_{22} & 0 & \cdots & 0 & 0 \\ 0 & -F_{32} & f_{33} & \cdots & 0 & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & 0 & \cdots & f_{n-1,n-1} & 0 \\ 0 & 0 & 0 & \cdots & -F_{n-1,n} & f_{nn} \\ 0 & 0 & 0 & \cdots & 0 & -F_{n+1,n} \end{bmatrix}$$

All quantities of the form  $F_{ij}$  and  $f_{ii}$  are strictly positive. Note that  $V$  is a rectangular  $(n+1) \times (n)$  matrix (i.e.  $V : \mathbb{R}^n \rightarrow \mathbb{R}^{n+1}$ ), while  $S$  is a rectangular  $(n) \times (n+1)$  matrix (i.e.  $S : \mathbb{R}^{n+1} \rightarrow \mathbb{R}^n$ ), and they have opposite sign structures.

The Jacobian  $J$  of the full system with the potential included is an  $(n+1) \times (n+1)$  matrix of the form:

$$\begin{bmatrix} -f_{11} - F_{21} & f_{22} & \cdots & 0 & F_2 - F_1 \\ F_{21} & -f_{22} - F_{32} & \cdots & 0 & F_3 - F_2 \\ \vdots & \vdots & \ddots & \vdots & \vdots \\ 0 & 0 & \cdots & -f_{nn} - F_{n+1,n} & F_{n+1} - F_n \\ P_1 f_{11} - P_2 F_{21} & P_2 f_{22} - P_3 F_{32} & \cdots & P_n f_{nn} - P_{n+1} F_{n+1,n} & -u + \sum_{i=1}^{n+1} (P_i F_i) \end{bmatrix}$$

Elementary physical assumptions imply that  $u > 0$ ,  $P \equiv [P_1, P_2, \dots, P_{n+1}]^T > 0$  and  $F \equiv [F_1, F_2, \dots, F_{n+1}]^T < 0$ . (Note that in the notation of [1]  $P_i = p_i$  and  $F_i = -F_{i\psi}$ .)

We wish to use Theorem 6.2 to show that  $J$  is a  $P^{(-)}$  matrix for all  $P > 0$ ,  $F < 0$  and  $u > 0$ . Incidentally this is hard to show by any direct method, but becomes almost immediate by Theorem 6.2. Note first that  $SV$  is of the form discussed in Appendix B, and thus  $SV$  is a nonsingular  $M^{(-)}$  matrix and hence a  $P^{(-)}$  matrix.

We start by showing that the sign of  $\det(J)$  is  $(-1)^{n+1}$ . Since  $\det(SV)$  has sign  $(-1)^n$ , it suffices to examine  $\ker(S)$  and  $\ker(V^T)$ . We can see that the  $\ker(S)$  consists only of multiples of the vector  $[1, 1, \dots, 1]^T$ . On the other hand, by inspection or induction, the strictly positive vector defined by:

$$y_1 = 1 \tag{6.3}$$

$$y_{i+1} = \frac{f_{i,i}}{F_{i+1,i}} y_i, \quad i = 1, \dots, n \tag{6.4}$$

spans  $\ker(V^T)$ . Thus this situation corresponds to the special case where  $\ker(S)$  lies entirely in the nonnegative and nonpositive orthants of  $\mathbb{R}^{n+1}$  and  $\ker(V^T)$  contains a strictly positive vector, confirming that  $\det(J)$  has sign  $(-1)^{n+1}$ .

We now show that  $J$  is a  $P^{(-)}$  matrix using Theorem 6.3. For any  $\alpha \subset \{1, \dots, n\}$ , the coordinates of a vector  $x \in \ker(S^\alpha)$  are defined by the equations

$$x_{i+1} = x_i, \quad i = 1, \dots, n \quad i \notin \alpha$$

On the other hand vectors  $y \in \ker((V^\alpha)^T)$  satisfy

$$y_{i+1} = \frac{f_{i,i}}{F_{i+1,i}} y_i, \quad i = 1, \dots, n \quad i \notin \alpha$$

Let  $x$  be an arbitrary vector in  $\ker(S^\alpha)$  with some coordinates  $x_j, \dots, x_{j+k}$  negative. Then regardless of the sizes of  $f_{ii}$  and  $F_{ij}$  we can choose a positive vector  $y \in \ker((V^\alpha)^T)$  with  $y_j, \dots, y_{j+k}$  much larger in magnitude than the other coordinates of  $y$  so that  $\langle x, y \rangle < 0$ . Thus the conditions of Theorem 6.3 are satisfied and the Jacobian is a  $P^{(-)}$  matrix.

**7. Conclusions and extensions.** We have shown that the structure of chemical reaction systems alone can determine whether their Jacobians are  $P^{(-)}$  matrices. The property of the stoichiometric matrix being SSD for general reaction systems, and WSD for mass action reactions, has been shown to be fundamentally linked to whether these systems can admit multiple equilibria. A technique has been presented to study when the  $P^{(-)}$  matrix property is preserved under rate-dependent negative feedback.

There are several possible extensions to this work. In the discussion on rate dependent negative feedback processes, an arbitrary row with particular sign structure was added to the stoichiometric matrix  $S$  and a column with opposite sign structure to the matrix  $V$ . However in the case where the extra row in  $S$  corresponds to a chemical reactant this row is a constant, and only the column added to  $V$  can vary. This situation clearly gives rise to less restrictive conditions on  $S$  and  $V$  which would preserve the  $P^{(-)}$  property of the Jacobian under the feedback. A related question is to find a *geometric* (rather than a combinatorial) characterisation of when adding a row (column) to a given SSD matrix preserves the SSD property. Finding such a characterisation would be helpful in explaining why many real reaction systems do have the SSD property.

The reader might have noted that during this paper we have nowhere used the law of atomic balance [7]. Intuitively we know that the two reactions  $A \rightleftharpoons B$  and  $A \rightleftharpoons 2B$  cannot both be true reactions (with no inflow or outflow involved). Mathematically this corresponds to the fact that the stoichiometric matrix  $S$  of the true reactions should have at least one (often more) nonnegative left eigenvectors of zero, corresponding to conserved quantities. This endows  $S$  with additional structure and it would be of interest to examine how this extra structure affects the likelihood of a given stoichiometric matrix being SSD.

We discussed the fact that certain additional assumptions can mean that  $P^{(-)}$  matrices are actually Hurwitz. One of the most interesting of these is sign-symmetry which can be implied by certain physical assumptions. We hope, in future work, to expand on these ideas as they form an interesting extension to the results in this paper.

On the same theme, when reaction systems have Jacobians with more structure than simply being a  $P^{(-)}$  matrix, and can be shown to be Hurwitz, it may sometimes

be possible to write down sufficient conditions which guarantee that the system with feedback remains Hurwitz. For example if the Jacobian of the full system is an  $H$  matrix [13], or is similar to one by a transformation preserving the  $M^{(-)}$  structure of the original system.

**Acknowledgements.** Thanks are due to the reviewer of this manuscript who helped us understand several connections between this work and the work in [5].

#### Appendix A. Properties of SSD and WSD matrices.

We collect a few easy results on SSD and WSD matrices which are needed for the arguments in this text. Note that by definition, any submatrix of an SSD (WSD) matrix is SSD (WSD). Note also that swapping rows/columns of a matrix does not alter whether it is SSD (WSD).

The first result is a trivial consequence of the definitions and the properties of determinants.

**LEMMA A.1.** *Let  $S$  be any square matrix. Multiply some column or row in  $S$  by a scalar constant to get a new matrix  $\tilde{S}$ . Then if  $S$  is SNS or singular, so is  $\tilde{S}$ .*

The next result states that it is possible to augment matrices in certain simple ways and preserve the SSD property.

**LEMMA A.2.** *Let  $S$  be an SSD matrix. Augment  $S$  with a single column (row) which is a scalar multiple of some column (row) of  $S$  to get a new matrix  $S_{aug}$ . Then  $S_{aug}$  is SSD.*

*Proof.* Any square submatrix of  $S_{aug}$  either

1. occurs in  $S$  in which case it is SNS or singular because  $S$  is SSD,
2. is a square submatrix of  $S$  with one column/row multiplied by a scalar in which case it is SNS or singular by Lemma A.1,
3. Contains some subset of both the original column/row and its multiple and is hence singular.  $\square$

Incidentally the above result would not hold if we replaced SSD by WSD: Although by definition any submatrix of a WSD matrix is WSD, the augmented versions of WSD matrices are not necessarily WSD. Thus in the statements of the Theorems on mass action systems in Section 4 it is essential that the systems be written as sets of irreversible reactions.

The next result shows that the set of WSD matrices contains the set of SSD matrices. (This is also a corollary of Theorem 1.2.5. in [4].)

**LEMMA A.3.** *Let  $S$  be a SNS or singular matrix and  $S_-$  be the matrix  $S$  with all positive entries set to zero. Then  $\det(S)\det(S_-) \geq 0$ .*

*Proof.* If  $S$  is singular the result is trivial, so assume that  $S$  is SNS. Consider the family of matrices  $S_p = (1-p)S + pS_-$  with  $p \in [0, 1]$ . By the definition of SNS, if  $S$  is SNS, then  $S_p$  is in the same qualitative class as  $S$  for  $p \in [0, 1]$ . By continuity of the determinant,  $S_-$  either has the same sign as  $S$  or is singular.  $\square$

From the previous two results it follows that if the stoichiometric matrix of a system of reactions is SSD, then it is also SSD when written as a system of irreversible reactions, in which case it is WSD when written this way.

The next few results are useful from an algorithmic point of view – they can considerably reduce the computational effort involved in calculating whether a matrix is SSD/WSD or not.

LEMMA A.4. *Let  $S$  be an SSD matrix. Let  $S_{aug}$  be the matrix  $S$  with a row/column containing at most one nonzero element added. Then  $S_{aug}$  is SSD.*

*Proof.* Any square submatrix of  $S_{aug}$  is either

1. a submatrix of  $S$ , and hence SNS or singular,
2. a single element and hence trivially SNS or singular, or
3. a submatrix of  $S$  augmented with an extra row/column containing at most one nonzero element. In this case the determinant is either zero or the product of a nonzero element and the determinant of a submatrix of  $S$  which is itself SNS or singular.  $\square$

From this it follows immediately that:

LEMMA A.5. *Let  $S$  be a matrix which is not SSD. Let  $S_{dim}$  be the matrix  $S$  with some rows/columns containing no more than one nonzero element removed. Then  $S_{dim}$  is not SSD.*

*Proof.* Suppose  $S_{dim}$  is SSD. A square submatrix of  $S$  is either

1. diagonal
2. a submatrix of  $S_{dim}$
3. a submatrix of  $S_{dim}$  augmented with rows/columns containing no more than one nonzero element.

In the first two cases it is trivial that the square submatrix is SNS or singular. In the third case the result follows from repeated application of Lemma A.4.  $\square$

Since any submatrix of an SSD matrix is SSD by definition, it follows that the full stoichiometric matrix of a CFSTR system (termed  $S_f$  in the text) is SSD iff the stoichiometric matrix  $S$  of the true reactions is SSD. In other words columns containing a single element (corresponding to inflow/outflow processes) can be ignored when checking if a matrix is SSD. Lemma A.5 also often considerably reduces the computational effort involved in checking if a matrix is SSD by allowing one to ignore rows in  $S$  containing a single element (i.e. to ignore reactants which participate in only one reaction).

The above result also extends to WSD matrices, and thus reduces the computational effort involved in checking whether a matrix which has been shown not to be SSD is actually WSD. The next lemma while tedious to state is actually very useful in practice.

LEMMA A.6. *Let  $S_r$  refer to the stoichiometric matrix of a system of reactions, and  $S_{ir}$  refer to the stoichiometric matrix of the system written as a set of irreversible reactions. Let  $\alpha$  be the set of rows in  $S_r$  containing a single element and  $\gamma$  be the set of columns in  $S_r$  containing a single element. Let  $S_{dim}$  be the matrix  $S_{ir}$  with rows from  $\alpha$  and columns from  $\gamma$  deleted. Then  $S_{ir}$  is WSD if and only if  $S_{dim}$  is.*

*Proof.* The ‘‘only if’’ part is trivial as  $S_{dim}$  is a submatrix of  $S_{ir}$ . Suppose  $S_{ir}$  is not WSD and consider a square submatrix  $T$  of  $S_{ir}$  which does not satisfy  $\det(T)\det(T_-) \geq 0$ . Any elements of  $T$  not in  $S_{dim}$  must lie rows/columns of  $T$  containing a single nonzero element, because if they lie in rows containing a single positive and a single negative element, then two columns of  $T$  will be multiples of each other and hence  $T$  will be singular. Further, any nonzero elements of  $T$  not in  $S_{dim}$  must be negative, since otherwise  $T_-$  would contain a row of zeros and thus be singular. The only way that  $\det(T)$  can be nonzero is if it takes the form of the product of these negative elements with the determinant of a submatrix  $\tilde{T}$  of  $S_{dim}$ . Similarly  $\det(T_-)$  must take the form of the product of these negative elements with

the determinant of  $\tilde{T}_-$ . Thus  $\det(T)\det(T_-)$  is a positive multiple of  $\det(\tilde{T})\det(\tilde{T}_-)$ , implying that  $\det(\tilde{T})\det(\tilde{T}_-) < 0$ . Thus  $S_{dim}$  is not WSD.  $\square$

This final lemma means that in checking whether a non-SSD matrix is actually WSD one can first remove rows corresponding to reactants which only occur in one (perhaps reversible) reaction from the stoichiometric matrix before checking the matrix.

### Appendix B. Binary reaction systems.

We present an important class of systems which give rise to  $P^{(-)}$  matrix Jacobians. Consider a set of  $n$  reactants  $A_i, i = 1, \dots, n$ . Assume the only reactions taking place are (reversible or irreversible) interconversions between the reactants along with some inflow and outflow processes. It is reasonable to assume that the rates depend on the substrates in a monotone way, so such systems are nonautocatalytic. They have been discussed in some detail in [2] where global stability of a unique equilibrium was shown using techniques connected with logarithmic norms. Each column of the stoichiometric matrix  $S$  either contains a  $+1$  and a  $-1$  (interconversion), or a single negative entry (outflow). It can be shown inductively that this structure implies that  $S$  is SSD and hence that the Jacobian is a  $P_0^{(-)}$  matrix.

Here we show that subject to a weak assumption on the inflow and outflow processes, the Jacobian  $J$  is a  $P^{(-)}$  matrix and in fact a nonsingular  $M^{(-)}$  matrix.

Associated with any such interconversion network is a directed graph  $\mathcal{G}$  on  $n + 1$  nodes. Nodes  $i = 1, \dots, n$  correspond to the  $n$  substrates, while the extra node which we term node 0 corresponds to the zero complex: i.e. to the outside of the system. For  $i, j \geq 1$ , there is an edge from node  $i$  to node  $j$  ( $i \neq j$ ) if and only if  $J_{ji} > 0$  – i.e.  $A_i$  can be converted to  $A_j$ , or alternatively the rate of conversion of  $A_j$  to  $A_i$  is inhibited by the concentration of  $A_i$ . On the other hand, for  $i \geq 1$ , there is an edge from node  $i$  to node 0 if and only if  $A_i$  is either subject to an outflow process or to an inflow process whose rate is inhibited by an increase in the concentration of  $i$ . This has the consequence of ensuring that  $J$  is strictly dominant in the  $i$ th column.

Our assumption is that **there is a directed path in  $\mathcal{G}$  from any node to node 0**. This has the physical interpretation that the concentration of any substrate is affected by the “outside” a considerably weaker condition than insisting on a CFSTR. We refer the reader to [2] for the details, but the above assumptions together imply that:

1.  $J$  has negative diagonal entries
2.  $J$  has nonnegative off-diagonal entries
3. There is a constant coordinate transformation  $T$  such that  $TJT^{-1}$  still satisfies conditions 1 and 2 and is also strictly diagonally dominant in every column; hence  $J$  is Hurwitz.

These three facts combine to ensure that  $J$  is a nonsingular  $M^{(-)}$  matrix [3]. Nonsingular  $M^{(-)}$  matrices are a subset of  $P^{(-)}$  matrices. In fact all trajectories converge to a unique equilibrium [2].

Note that the basic system of coupled redox reactions presented in [1] gives rise to a Jacobian of the above form, even though in this case electrons are being transferred rather than reactants interconverted.

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