Multiple equilibria in complex chemical reaction networks: extensions to entrapped species models

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Abstract: In two earlier papers, means were provided to decide the capacity of complex chemical reaction networks, taken with mass-action kinetics, to admit multiple equilibria in the context of the isothermal homogeneous continuous flow stirred tank reactor (CFSTR). In such a reactor, all species are deemed to be in the outflow, a fact which has an important bearing on the nature of the governing equations. In contrast, one can imagine CFSTR-like models of the cell in which certain large molecules (e.g. enzymes) remain entrapped within the cell, whereas smaller ones (e.g. metabolites) are free to diffuse through the cell boundary. Although such models bear a strong physical resemblance to the classical CFSTR picture, there are substantive differences in the corresponding mathematics. Without a presumption of mass-action kinetics, this research is intended to indicate a general way in which results about uniqueness of equilibria in the classical CFSTR context extend to entrapped species models.

1 Introduction

In two earlier papers [1, 2] we developed means to determine whether a given complex reaction network, taken with mass-action kinetics, has the capacity to exhibit more than one steady state. That is, our interest was in whether, for the network, there exist parameter values such that the corresponding isothermal mass-action differential equations admit at least two distinct rest points. (This is surprisingly uncommon.) In the first paper, the theory led to a test that lends itself to computational implementation, whereas in the second paper, the capacity for multiple steady states was tied more directly to subtle aspects of reaction network structure, as revealed in the network's species-reaction graph. (At the end of this section we shall state a theorem, which is a consequence of results in Craciun and Feinberg [2] and that connects the species-reaction graph to the capacity for multiple steady states.)

In both papers, it was understood that the reactions were taking place in the context of what chemical engineers call a continuous flow stirred tank reactor (CFSTR). In particular, the reacting mixture was presumed to be an incompressible liquid, filling a perfectly stirred vessel maintained at constant temperature and volume. (We assume isothermal incompressible mixtures throughout.) Moreover, it was supposed that at least certain reactants were fed to the vessel at constant rate and that all species were removed from the vessel at rates proportional to their molar concentrations within the vessel. (In the normal chemical engineering context, mixture is withdrawn from the vessel at a constant volumetric flow rate, which is identical to the volumetric flow rate of the feed stream. The molar removal rate of a particular species, therefore, is just the volumetric flow rate of the outflow stream multiplied by the molar per unit volume concentration of that species within the vessel.)

Our aim here is to extend results in the earlier papers to settings that are only slightly different physically from the classical CFSTR but that are substantively different mathematically. In particular, we want to consider variants of the CFSTR in which only certain species are in the outflow. We are motivated by consideration of CFSTR-like models for the cell in which biochemical reactions are driven by enzyme-catalysis. The presumption is that the enzyme(s) remains within the cell, neither entering it nor leaving it, while playing their catalytic role in the cell's interior repeatedly. In contrast, small metabolites are free to cross the cell boundary, playing the role of substrates and products of the various enzyme-catalysed reactions. We presume, as in earlier work [1, 2], that the mixture within the cell remains spatially homogeneous. Of course, this model for the cell is only a metaphor, but, given the complexities of most real biochemical networks, it is a metaphor that at least serves to isolate sources of dynamical behaviour that have their origins in the chemistry itself. (Although we shall have in mind the image of the CFSTR-like cell, the same mathematics serves to describe intracellular biochemical modules [3, 4] in which certain species are synthesised at constant rate, while certain species are degraded at rates proportional to their concentrations. In such cases, constant-rate species synthesis plays the role of transport to the cell, while species degradation plays the role of transport from the cell.)

To understand the substantive technical differences between the 'entrapped enzyme' picture and the situation in which all species are free to leave the cell, it will be useful to consider the very simplest (mass-action) model of enzyme catalysis, depicted in (1). The substrate S binds to an enzyme E to form an enzyme–substrate complex SE, from which the product P is released, while the enzyme returns to its original state. Suppose that these reactions

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occur within a spatially homogeneous cell immersed in a time-invariant ambient medium and that, in the medium, concentrations of the species are maintained at fixed values c_S^0, c_E^0, c_{SE}^0 and c_P^0. We denote by c_S, c_E, c_{SE} and c_P the concentrations of the species in the cell's interior.

$$S + E \rightleftarrows SE \rightarrow P + E \tag{1}$$

To begin, imagine that every species is free to diffuse through the boundary of the cell and that the net molar transfer rate of each species to the cell from the ambient medium is proportional to the concentration difference of that species across the boundary. Thus, for example, the net molar transfer rate (per unit cell volume) of species S to the cell from the ambient medium is $\alpha_S(c_S^0 - c_S)$, where α_S is a mass transfer coefficient for species S. Taking into account the contributions of mass transfer and chemical reactions, we write the differential equations governing the species concentrations within the cell as in (2), where $k_{S+E\rightarrow SE}$, $k_{SE\rightarrow S+E}$ and $k_{SE\rightarrow P+E}$ are the mass-action rate constants for the corresponding reactions. (Note that equations (2) become those of the classical CFSTR if all the mass transfer coefficients are set to g/V, where V is the volume of the reacting mixture and g the volumetric flow rate of the outflow and feed streams.)

$$\begin{aligned}
\dot{c}_S &= \alpha_S(c_S^0 - c_S) - k_{S+E\rightarrow SE}\, c_S c_E + k_{SE\rightarrow S+E}\, c_{SE} \\
\dot{c}_E &= \alpha_E(c_E^0 - c_E) - k_{S+E\rightarrow SE}\, c_S c_E \\
&\quad + (k_{SE\rightarrow S+E} + k_{SE\rightarrow P+E})c_{SE} \\
\dot{c}_{SE} &= \alpha_{SE}(c_{SE}^0 - c_{SE}) + k_{S+E\rightarrow SE}\, c_S c_E \\
&\quad - (k_{SE\rightarrow S+E} + k_{SE\rightarrow P+E})c_{SE} \\
\dot{c}_P &= \alpha_P(c_P^0 - c_P) + k_{SE\rightarrow P+E}\, c_{SE}
\end{aligned} \tag{2}$$

To ask whether, in the context described, network (1) *has the capacity for multiple positive equilibria* is to ask whether there are positive values for the rate constants, mass transfer coefficients and ambient concentrations such that the system of polynomial equations, obtained by setting the time derivatives in (2) to zero, admit two or more distinct positive solutions for c_S, c_E, c_{SE} and c_P. In fact, theory from Craciun and Feinberg [1, 2] indicates very quickly that (2) does not have the capacity for multiple positive equilibria. (See the theorem statement at the end of this section. The species-reaction graph has a single cycle, and it is a 1-cycle.)

Now consider the 'entrapped enzyme' picture. That is, suppose that the cell boundary is impermeable to species E and ES. In this case, the governing differential equations become those shown in (3). Note that the equations for equilibria, obtained by setting the time derivatives to zero, become redundant; in particular, the second and third (equilibrium) equations are identical up to a change in sign. Thus, there are essentially three equilibrium equations to determine the four equilibrium concentrations, c_S, c_E, c_{SE} and c_P. In contrast to the situation in (2), there are now an uncountable number of positive equilibria, no matter what positive values the parameters take.

$$\begin{aligned}
\dot{c}_S &= \alpha_S(c_S^0 - c_S) - k_{S+E\rightarrow SE}\, c_S c_E + k_{SE\rightarrow S+E}\, c_{SE} \\
\dot{c}_E &= -k_{S+E\rightarrow SE}\, c_S c_E + (k_{SE\rightarrow S+E} + k_{SE\rightarrow P+E})c_{SE} \\
\dot{c}_{SE} &= k_{S+E\rightarrow SE}\, c_S c_E - (k_{SE\rightarrow S+E} + k_{SE\rightarrow P+E})c_{SE} \\
\dot{c}_P &= \alpha_P(c_P^0 - c_P) + k_{SE\rightarrow P+E}\, c_{SE}
\end{aligned} \tag{3}$$

This, however, is to be expected on physical grounds. Note that from (3), we have $\dot{c}_E + \dot{c}_{SE} = 0$ so, for all t, $c_E(t) + c_{SE}(t) = c_E(0) + c_{SE}(0)$. That is, the total amount of enzyme (either with or without S bound to it) remains equal to its initial supply in the cell. Thus, two different initial supplies of enzyme in the cell cannot possibly result in the same equilibrium. The appropriate uniqueness question, then, becomes this: For a given supply of enzyme (i.e. for a given value of $c_E(0) + c_{SE}(0)$), can there be more than one positive equilibrium? In assessing network (1)'s capacity for multiple positive equilibria in the entrapped enzyme context, we would now ask: Are there positive values of the mass transfer coefficients, the rate constants, c_S^0, c_P^0, and a value of the total enzyme concentration such that two or more distinct equilibria corresponding to this enzyme supply are admitted by (3)?

It should be clear, then, that there is a difference in questions appropriate to the 'entrapped enzyme' picture and the picture in which all species are permitted to diffuse through the cell boundary (the 'fully diffusive' picture). Some care is required in the passage from results about one situation to assertions about the other.

Some of the most important results in Craciun and Feinberg [1, 2] are of the kind that assert that certain highly complex mass-action networks do not have the capacity for multiple positive equilibria when all species are permitted to cross the cell boundary. That is, no matter what the parameter values are, the corresponding differential equations (exemplified by (2)) cannot admit more than one positive equilibrium. Note that these are assertions about the reaction network that gives rise to the corresponding equations, for when the kinetics is mass-action the network itself determines the shape of the equations up to parameter values.

We would like to assert that, with very slight modification in their statements, these results also serve to preclude multiple positive equilibria for a wide variety of intricate enzymatic networks in the context of the 'entrapped enzyme' picture, provided that uniqueness of equilibria is construed in the sense described earlier. That is, we consider uniqueness of equilibria consistent with fixed supplies of enzyme(s). Our aim in this article is to show that, under the very same hypotheses, results in Craciun and Feinberg [1, 2] do indeed carry over unchanged to preclude multiple positive equilibria in the entrapped enzyme context, except perhaps for multiple equilibria of a highly degenerate nature. In this way, broad theory about highly complex reaction networks becomes extensible to settings more suited for cell biology. (The ideas underlying the extension are not restricted to mass-action kinetics.)

For the benefit of readers unfamiliar with results in Craciun and Feinberg [2], we conclude this section by providing a brief discussion of the kind of statements those results permit one to make. (The sample theorem provided here is, in fact, a special case of a more general version.)

Let us consider the following enzymatic reaction network, involving two enzymes, E1 and E2

$$\begin{aligned}
&S1 + E1 \rightleftarrows E1S1 \\
&S2 + E1S1 \rightleftarrows E1S1S2 \rightarrow P1 + E1 \\
&S2 + E2 \rightleftarrows E2S2 \rightarrow 2S1 + E2
\end{aligned} \tag{4}$$

In the context of mass-action kinetics, and if we assume that every species is free to diffuse through the boundary of the cell, reaction network (4) does not have the capacity for multiple positive equilibria. One way to check this is to use a species-reactions graph criterion, as described in Craciun and Feinberg [2]. The species reaction graph of this reaction network is shown in Fig. 1.

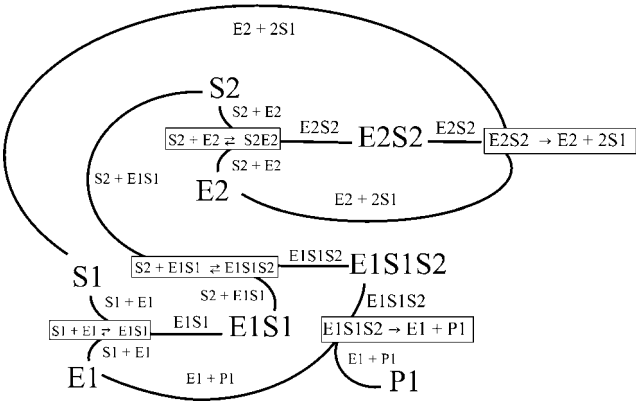

Fig. 1 *The species-reaction graph for network (4)*

In general, the species-reaction graph of a reaction network is a bipartite graph [5]. The *nodes* of the graph are either species nodes or reaction nodes; the graph has one node for each species and one node for each (reversible or irreversible) reaction. The *complexes* of a reaction network are the objects before and after the reaction arrows. (For example, S1 + E1 and E1S1 are both complexes.) Each *edge* of the graph connects a species node and a reaction node if that species appears in a complex of that reaction; moreover, we label each edge with that complex. For example, there is an edge connecting the species node S1 and the reaction node S1 + E1 $\rightleftarrows$ E1S1 because the species S1 appears in the complex S1 + E1, and we label this edge with the complex S1 + E1.

In order to be able to present the statement of a theorem implying that the reaction network (4) does not have the capacity for multiple positive equilibria, we need to define some features of species-reaction graphs. Pairs of edges that meet at a reaction node and have the same complex label are called *c-pairs* (abbreviation for complex-pair). For example, the two edges labelled S1 + E1 form a c-pair. Note that cycles may appear in species-reaction graphs. Cycles that contain an odd number of c-pairs are called *odd-cycles*, and cycles that contain an even number of c-pairs are called *even-cycles*. The stoichiometric coefficient of an edge is the coefficient of the species adjacent to that edge in the complex label of the edge. Cycles for which all edges have *stoichiometric coefficient* equal to one are called *1-cycles*. (Note that a 1-cycle will also be either an even-cycle or an odd-cycle.) We say that two cycles *split a c-pair* if each edge of the c-pair appears in at least one of the cycles, and one of the cycles contains only one edge of the c-pair (while the other cycle might contain the other edge, or both). For example, the cycle that passes through the species nodes E1, E1S1 and E1S1S2, and the cycle that passes through the species nodes E1S1, S2, E2S2, S1, split the c-pair labelled S1 + E1.

The following theorem follows from Craciun and Feinberg [2]. It is understood that the 'capacity for multiple positive equilibria' refers to the CFSTR equations, formulated as in, corresponding to the case in which all species are permitted passage through the reactor boundary.

Theorem 1: Consider a reaction network such that, in its species-reaction graph, all cycles are odd-cycles or 1-cycles, and no two even-cycles split a *c*-pair. Then, taken with mass-action kinetics, the reaction network does not have the capacity for multiple positive equilibria (regardless of values of the rate constants, mass transfer coefficients or ambient concentrations).

It can be confirmed without much difficulty that all cycles in Fig. 1 are odd-cycles or 1-cycles (or both). Moreover, there are only two 1-cycles that are also even-cycles: the one that passes through the species nodes E1, E1S1 and E1S1S2, and the one that passes through the species nodes E2 and E2S2. These two even-cycles do not split a c-pair, as they do not have any nodes in common. Therefore, according to Theorem 1, the reaction network, taken with mass-action kinetics, does not have the capacity for multiple positive equilibria (in the fully diffusive case).

Criteria such as Theorem 1 provide powerful and very delicate necessary conditions that a network must satisfy in order to have the capacity for multiple positive equilibria. (At least one of its easily satisfied species-reaction graph requirements must be violated.) Other such results are described in Craciun and Feinberg [1, 2]. They rely on the assumption that every species is free to diffuse through the boundary of the cell. Our goal is to extend the applicability of these methods to the case where some species are entrapped within the cell.

Before proceeding, we note that readers of this article might find useful a monograph by Érdi and Tóth [6], which surveys other work connecting chemical dynamics to reaction network structure, some of it having a graphical character. For a discussion of connections between multiple equilibria and oscillations, see also Tóth [7].

2 Reaction networks and kinetic systems

We follow the general scheme in Craciun and Feinberg [1, 2], and Feinberg [8, 9] for describing chemical reaction networks and their kinetics. The real numbers are denoted by $\mathbb{R}$, the positive real numbers by $\mathbb{R}_+$ and the non-negative real numbers by $\overline{\mathbb{R}}_+$. If I is a finite index set, we denote by $\mathbb{R}^I$ the vector space of all formal (real) linear combinations of I. Thus, an element $x \in \mathbb{R}^I$ has a representation of the form

$$x = \sum_{i \in I} x_i i$$

with $x_i \in \mathbb{R}$. The support of an element $x \in \mathbb{R}^I$ (denoted supp x) is the set of all $i \in I$ such that $x_i \neq 0$. By $\mathbb{R}^I_+$ [respectively $\overline{\mathbb{R}}^I_+$] we mean the set of $x \in \mathbb{R}^I$ for which $x_i > 0$ [respectively $x_i \geq 0$], for all $i \in I$. We give $\mathbb{R}^I$ the scalar product (and resulting norm topology) defined by

$$x.y := \sum_{i \in I} x_i y_i$$

By the complexes in a reaction network, we mean the formal linear combinations of the species that appear at the heads and tails of the reaction arrows – for example, S + E, SE and P + E in network (1). Thus, if $\mathcal{S}$ is the set of species in a network (e.g. {S, E, SE, P} in (1)), then the complexes of the network are elements of $\overline{\mathbb{R}}^{\mathcal{S}}_+$. The reactions of a network are then specified by a 'reacts to' relation in the set of complexes. With this as background, we define a reaction network as follows.

Definition 1: A reaction network is specified by a triplet $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$, where

(i) $\mathcal{S}$ is a finite set of species
(ii) $\mathcal{C} \subset \overline{\mathbb{R}}^{\mathcal{S}}_+$ is a finite set of complexes
(iii) $\mathcal{R} \subset \mathcal{C} \times \mathcal{C}$ is a 'reacts to' relation in $\mathcal{C}$ such that

(a) $(y, y) \notin \mathcal{R}, \quad \forall y \in \mathcal{C}$
(b) for each $y \in \mathcal{C}$, $\exists\, y' \in \mathcal{C}$ such that $(y, y') \in \mathcal{R}$ or $(y', y) \in \mathcal{R}$.

Elements of $\mathcal{R}$ are the reactions of the network. We write the more suggestive $y \to y'$ in place of (y, y') if and only if $(y, y') \in \mathcal{R}$.

A *composition* for a mixture with species set $\mathcal{S}$ is a specification of a molar concentration c_s for each $s \in \mathcal{S}$. Thus, we can identify a composition with an element $c \in \overline{\mathbb{R}}_+^{\mathcal{S}}$. A *kinetics* for a reaction network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ is an assignment to each reaction $y \to y' \in \mathcal{R}$ of a non-negative-real-valued *rate function* $\mathcal{K}_{y\to y'}(\cdot)$ with domain $\overline{\mathbb{R}}_+^{\mathcal{S}}$. For each composition $c \in \overline{\mathbb{R}}_+^{\mathcal{S}}$, $\mathcal{K}_{y\to y'}(c)$ is interpreted as the molar occurrence rate per unit volume of reaction $y \to y'$ when the mixture has composition c. Hereafter, we suppose that rate functions are continuously differentiable on $\mathbb{R}_+^{\mathcal{S}}$. (Although it will not be important to this article, it is natural to require that, for each $y \to y'$ in $\mathcal{R}$, $\mathcal{K}_{y\to y'}(c)$ be strictly positive precisely when supp $y \subset$ supp c – that is, precisely when the composition c contains at non-zero concentrations those species that appear in the reactant complex y.) By a *kinetic system*, which we indicate symbolically as $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K}\}$, we mean a reaction network taken together with a kinetics.

Example 1: A mass-action kinetics for a reaction network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ is a kinetics having the following property: For each $y \to y' \in \mathcal{R}$, there is a positive rate constant $k_{y\to y'}$ such that

$$\mathcal{K}_{y\to y'}(c) \equiv k_{y\to y'} \prod_{s\in\mathcal{S}} c_s^{y_s} \tag{5}$$

Note that a mass-action kinetics for a network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$ is specified completely by an assignment to each reaction of a positive rate constant, so we can identify a particular mass-action kinetics with an element $k \in \mathbb{R}_+^{\mathcal{R}}$. With this in mind, we shall sometimes refer to the mass-action system $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, k\}$.

Definition 2: For a kinetic system $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K}\}$ the species formation rate function r: $\overline{\mathbb{R}}_+^{\mathcal{S}} \to \mathbb{R}^{\mathcal{S}}$ is defined by

$$r(c) = \sum_{y\to y'\in\mathcal{R}} \mathcal{K}_{y\to y'}(c)(y' - y) \tag{6}$$

The interpretation of $r(\cdot)$ is as follows: In a mixture of composition c, $r_s(c)$ is the molar production rate per unit volume of species s due to the occurrence of all chemical reactions. To see this, note that

$$r_s(c) = \sum_{y\to y'\in\mathcal{R}} \mathcal{K}_{y\to y'}(c)(y'_s - y_s) \tag{7}$$

and that $y'_s - y_s$ is the net number of molecules of species s produced with each occurrence of reaction $y \to y'$. Thus, the right side of (7) is the sum of all the per unit volume reaction occurrence rates, each weighted by the net gain in molecules of s with each occurrence of the corresponding reaction.

Note that, for a kinetic system $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K}\}$, the species formation rate function takes values in the span of the set

$$\{y' - y \in \mathbb{R}^{\mathcal{S}} : y \to y' \in \mathcal{R}\} \tag{8}$$

Elements of the set (8) are the *reaction vectors* of the network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$. The *stoichiometric subspace* for a reaction network, which we denote by $\boldsymbol{S}$, is the span of its reaction vectors:

$$\boldsymbol{S} := \text{span}\{y' - y \in \mathbb{R}^{\mathcal{S}} : y \to y' \in \mathcal{R}\}. \tag{9}$$

For a kinetic system $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K}\}$, in the context of a well-stirred mixture filling a constant-volume cell for which there is no mass transfer to or from the cell, the differential equations governing the species concentrations reduce to

$$\dot{c} = r(c), \tag{10}$$

where $r(\cdot)$ is the species-formation rate function. Note that, in this context, $\dot{c}$ invariably points along the stoichiometric subspace for the underlying reaction network.

3 The entrapped species model

Now we consider the situation in which enzymes (and enzyme-bound substances such as SE in Section 1) are entrapped within the cell, while all other species (small metabolites) are free to diffuse across the cell boundary. To be somewhat more general, we suppose only that, for the operative reaction network $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$, the species set $\mathcal{S}$ is partitioned into two subsets, $\mathcal{E}$ and $\mathcal{M}$, called the *entrapped species* and the *mobile species*, respectively. For the application we have in mind, we envision $\mathcal{E}$ to be the enzymatic species and $\mathcal{M}$ to be the small metabolites, but this will not be important for mathematics. Rather, the species subset $\mathcal{E}$ can be construed simply as the set of all members of $\mathcal{S}$ denied passage through the cell boundary, while $\mathcal{M}$ is the complement of $\mathcal{E}$ in $\mathcal{S}$. For the simple entrapped enzyme example described in Section 1, we have $\mathcal{E} = \{\text{E, SE}\}$ and $\mathcal{M} = \{\text{S, P}\}$.

Hereafter, when we speak of an entrapped species model, it will be understood that there is a specified partition of the species set $\mathcal{S}$ into two subsets $\mathcal{E}$ and $\mathcal{M}$. We denote by $\Gamma_{\mathcal{E}}$ and $\Gamma_{\mathcal{M}}$ the linear subspaces of $\mathbb{R}^{\mathcal{S}}$ consisting of vectors having supports in $\mathcal{E}$ and $\mathcal{M}$, respectively. That is,

$$\Gamma_{\mathcal{E}} := \{x \in \mathbb{R}^{\mathcal{S}} : i \notin \mathcal{E} \Rightarrow x_i = 0\} \quad \text{and}$$

$$\Gamma_{\mathcal{M}} := \{x \in \mathbb{R}^{\mathcal{S}} : i \notin \mathcal{M} \Rightarrow x_i = 0\}.$$

Note that $\mathbb{R}^{\mathcal{S}} = \Gamma_{\mathcal{E}} \oplus \Gamma_{\mathcal{M}}$. We denote by $\text{P}_{\mathcal{E}}$: $\mathbb{R}^{\mathcal{S}} \to \Gamma_{\mathcal{E}}$ and $\text{P}_{\mathcal{M}}$: $\mathbb{R}^{\mathcal{S}} \to \Gamma_{\mathcal{M}}$ the projections onto $\Gamma_{\mathcal{E}}$ and $\Gamma_{\mathcal{M}}$, respectively.

Consider an entrapped species model that derives from a chemistry specified by the kinetic system $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K}\}$, with $\mathcal{S} = \mathcal{E} \sqcup \mathcal{M}$. Formulation of the corresponding entrapped species differential equations (analogous to (3)) requires specification of certain additional parameters apart from the kinetics – in particular, a mass transfer coefficient $\alpha_m > 0$ and an ambient molar concentration $c_m^0 \geq 0$ for each species $m \in \mathcal{M}$. In effect, then, specification of an entrapped species model amounts to specification of a kinetic system $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K}\}$, a partition of $\mathcal{S}$ into $\mathcal{E}$ and $\mathcal{M}$, and specification of two elements $\alpha \in \mathbb{R}_+^{\mathcal{M}}$ and $c^0 \in \overline{\mathbb{R}}_+^{\mathcal{M}}$.

Taking into account both chemical reactions and diffusive fluxes across the cell boundary, the entrapped species model differential equations governing the concentrations within the cell become

$$\dot{c} = g(c) \tag{11}$$

where $g(\cdot)$: $\overline{\mathbb{R}}_+^{\mathcal{S}} \to \mathbb{R}^{\mathcal{S}}$ is defined by

$$g(c) := r(c) + \sum_{m\in\mathcal{M}} \alpha_m(c_m^0 - c_m)m$$

$$= \sum_{y\to y'\in\mathcal{R}} \mathcal{K}_{y\to y'}(c)(y' - y) + \sum_{m\in\mathcal{M}} \alpha_m(c_m^0 - c_m)m \tag{12}$$

Note that $g(\cdot)$ takes values in the linear subspace

$$\bar{\boldsymbol{S}} := \text{span}(\{y' - y \in \mathbb{R}^{\mathcal{S}} : y \to y' \in \mathcal{R}\} \cup \mathcal{M}) = \boldsymbol{S} + \Gamma_{\mathcal{M}} \tag{13}$$

For the entrapped species model, then, $\dot{c}$ is no longer constrained to lie in the stoichiometric subspace for the underlying reaction network (as in the closed cell situation). Rather, $\dot{c}$ is constrained to lie in the somewhat larger linear subspace $\bar{\boldsymbol{S}}$. Still, $\bar{\boldsymbol{S}}$ will typically remain a proper subspace of $\mathbb{R}^{\mathcal{S}}$, for the reaction vectors will normally reflect certain intracellular conservation conditions among the entrapped (enzymatic) species – conditions that remain operative despite the transport of the mobile species (metabolites) across the cell boundary.

Example 2: Consider the simple entrapped enzyme example discussed in Section 1. For network (1), the reaction vectors are

$$\{\text{ES} - \text{E} - \text{S}, \quad \text{E} + \text{S} - \text{ES}, \quad \text{P} + \text{E} - \text{ES}\}$$

Moreover, $\mathcal{E} = \{\text{E}, \text{ES}\}$ and $\mathcal{M} = \{\text{S}, \text{P}\}$. Thus

$$\bar{\boldsymbol{S}} := \text{span}\{\text{ES} - \text{E} - \text{S}, \quad \text{E} + \text{S} - \text{ES}, \quad \text{P} + \text{E} - \text{ES}, \quad \text{S}, \quad \text{P}\} \subset \mathbb{R}^{\mathcal{S}}$$

Although $\dim \mathbb{R}^{\mathcal{S}} = 4$, it is not difficult to see that $\dim \bar{\boldsymbol{S}} = 3$, so $\bar{\boldsymbol{S}}$ is a proper linear subspace of $\mathbb{R}^{\mathcal{S}}$. In fact, it is apparent that $\text{E} + \text{ES} \in \mathbb{R}^{\mathcal{S}}$ is orthogonal to $\bar{\boldsymbol{S}}$. This orthogonality, taken together with the fact that $\dot{c}$ takes values in $\bar{\boldsymbol{S}}$, imply that $\dot{c}_{\text{E}} + \dot{c}_{\text{ES}} = 0$, which in turn reflects the constancy of $c_{\text{E}} + c_{\text{ES}}$ along solutions of (11).

Because $\dot{c}$ invariably points along $\bar{\boldsymbol{S}}$, it is not difficult to see that a composition c' can evolve from a composition c only if $c' - c$ is contained in $\bar{\boldsymbol{S}}$. From (13), it follows that this condition is satisfied precisely when $P_{\mathcal{E}}(c' - c) \in P_{\mathcal{E}}(\boldsymbol{S})$, the latter being the projection of the stoichiometric subspace into $\Gamma_{\mathcal{E}}$. This is to say that the composition change reflected in $c' - c$ must, for the entrapped species, derive only from the occurrence of chemical reactions. These considerations motivate the following definition.

Definition 3: Consider an entrapped species model in which the underlying reaction network is $\{\mathcal{S}, \mathcal{C}, \mathcal{R}\}$, with $\mathcal{S} = \mathcal{E} \sqcup \mathcal{M}$, and let $\bar{\boldsymbol{S}}$ be as in (13). Two compositions c and c' in $\overline{\mathbb{R}}^{\mathcal{S}}_+$ are entrapped species compatible (denoted $c \simeq c'$) if $c' - c$ lies in $\bar{\boldsymbol{S}}$. The equivalence relation $\simeq$ serves to partition $\overline{\mathbb{R}}^{\mathcal{S}}_+$ into entrapped species compatibility classes. (These are parallels of $\bar{\boldsymbol{S}}$, restricted to $\overline{\mathbb{R}}^{\mathcal{S}}_+$.).

Compositions along a solution of (11) that begins within a particular entrapped species compatibility class lie entirely within the same compatibility class. Indeed, each such compatibility class is the union of composition trajectories, and one can associate a flow, deriving from (11), with each class. Each of the various compatibility classes will usually have one or more equilibria of its own. Thus, questions about the existence of multiple equilibria should properly be construed as questions about the existence of more than one equilibrium within a compatibility class.

An equilibrium of a vector field is often said to be degenerate if the derivative of the vector field at that equilibrium is singular. Without some qualification, every positive equilibrium of (11) would typically be degenerate for the following reason: an equilibrium composition, say c^*, within a particular compatibility class will typically lie on a manifold of equilibria, each nearby point of which corresponds to an equilibrium within a different (nearby) compatibility class. Thus, $dg(c^*)$, the derivative of g at c^*, will be singular, for it will have in its kernel a vector tangent to the equilibrium manifold at c^*. For the situation at hand, then, a more appropriate notion of degeneracy would require that the singularity correspond to directions along the compatibility class containing c^*, not transverse to it. With this in mind we posit the following definition:

Definition 4: For an entrapped species model, an equilibrium $c^* \in \mathbb{R}^{\mathcal{S}}_+$ of (11) is non-degenerate if $(\ker dg(c^*)) \cap \bar{\boldsymbol{S}} = \{0\}$; otherwise, c^* is degenerate. We say that the model admits multiple non-degenerate equilibria if there are at least two distinct non-degenerate equilibria, say c^* and $c^{**} \in \mathbb{R}^{\mathcal{S}}_+$, such that $c^{**} - c^* \in \bar{\boldsymbol{S}}$. (The last requirement ensures that c^* and c^{**} are entrapped species compatible.)

4 The fully diffusive model

Here, we consider a chemistry described by the kinetic system $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K}\}$, but now we imagine that all species are permitted passage through the cell boundary. For an ambient-medium composition $\bar{c}_0 \in \overline{\mathbb{R}}^{\mathcal{S}}_+$ and a specification of mass transfer coefficients $\bar{\alpha} \in \mathbb{R}^{\mathcal{S}}_+$, the fully diffusive model differential equations, analogous to (2), take the form

$$\dot{c} = h(c) \tag{14}$$

where

$$h(c) := \sum_{y \to y' \in \mathcal{R}} \mathcal{K}_{y \to y'}(c)(y' - y) + \sum_{\mathcal{S} \in \mathcal{S}} \bar{\alpha}_{\mathcal{S}}(\bar{c}^0_{\mathcal{S}} - c_{\mathcal{S}})\mathcal{S} \tag{15}$$

In contrast to the situation for the entrapped species model, the range of $h(\cdot)$ will not usually be contained in a proper linear subspace of $\mathbb{R}^{\mathcal{S}}$. In this case, there will often be just one equilibrium in all of $\mathbb{R}^{\mathcal{S}}_+$, and, when there are more than one positive equilibrium, these will typically be few in number. This is different from the entrapped species model, for which manifolds of equilibria typically pass through the different entrapped species compatibility classes transversely.

5 A relationship between the entrapped species model and the fully diffusive model

Our aim in this section is to prove a theorem that will extend results in Craciun and Feinberg [1, 2] to entrapped species models.

Theorem 2: Suppose that, for a kinetic system $\{\mathcal{S}, \mathcal{C}, \mathcal{R}, \mathcal{K}\}$, there are no values of $\bar{c}^0 \in \overline{\mathbb{R}}^{\mathcal{S}}_+$ and $\bar{\alpha} \in \mathbb{R}^{\mathcal{S}}_+$ such that the fully diffusive differential equations (14) and (15) admit multiple positive equilibria. Then, for any specified partition $\mathcal{S} = \mathcal{E} \sqcup \mathcal{M}$, there are no values $\alpha \in \mathbb{R}^{\mathcal{M}}_+$ and $c^0 \in \overline{\mathbb{R}}^{\mathcal{M}}_+$ such that the entrapped species differential equations (11) and (12) admit multiple non-degenerate (positive) equilibria in the sense of Definition 4.

Proof: Suppose, on the contrary, that for $\alpha \in \mathbb{R}^{\mathcal{M}}_+$ and $c^0 \in \overline{\mathbb{R}}^{\mathcal{M}}_+$ (11) and (12) admit multiple non-degenerate equilibria. That is, suppose that there are $c^*, c^{**} \in \mathbb{R}^{\mathcal{S}}_+$ such that

$$c^{**} - c^* \in \bar{\boldsymbol{S}}, \tag{16}$$

$$g(c^*) = 0, \qquad g(c^{**}) = 0, \tag{17}$$

$$(\ker dg(c^*)) \cap \bar{\boldsymbol{S}} = \{0\}, \quad \text{and} \quad (\ker dg(c^{**})) \cap \bar{\boldsymbol{S}} = \{0\}. \tag{18}$$

Let $c^*_{\mathcal{E}}$ be the projection of c^* into $\Gamma_{\mathcal{E}}$. Because $\text{supp}(c^* - c^*_{\mathcal{E}})$ is contained in $\mathcal{M}$ and because $\Gamma_{\mathcal{M}}$ is contained in $\bar{\boldsymbol{S}}$, it follows that $c^* - c^*_{\mathcal{E}}$ is contained in $\bar{\boldsymbol{S}}$. Now, we define $\tilde{h}(\cdot,\cdot)$: $\mathbb{R}^{\mathcal{S}}_+ \times \mathbb{R} \to \mathbb{R}^{\mathcal{S}}$ by

$$\begin{aligned}\tilde{h}(c,\theta) &:= g(c) + \theta \sum_{e\in\mathcal{E}} (c^*_e - c_e)e \\ &= \sum_{y\to y'\in\mathcal{R}} \mathcal{K}_{y\to y'}(c)(y'-y) \\ &\quad + \sum_{m\in\mathcal{M}} \alpha_m(c^0_m - c_m)m + \theta\sum_{e\in\mathcal{E}}(c^*_e - c_e)e\end{aligned}$$

Our aim will be to show that there is a $\theta^\dagger > 0$ and distinct $\tilde{c}^*$ and $\tilde{c}^{**}$ such that $\tilde{h}(\tilde{c}^*, \theta^\dagger) = 0$ and $\tilde{h}(\tilde{c}^{**}, \theta^\dagger) = 0$. This will contradict the hypothesis that the fully diffusive model does not have the capacity for multiple equilibria. (In particular, the distinct positive equilibria $\tilde{c}^*$ and $\tilde{c}^{**}$ will correspond to the fully-diffusive-model parameter values $\bar{\alpha}_m = \alpha_m$, $\bar{c}^0_m = c^0_m$ for all $m \in \mathcal{M}$ and $\bar{c}^0_e = c^*_e$, $\bar{\alpha}_e = \theta^\dagger$, for all $e \in \mathcal{E}$).

To show the contradiction, we first let Ω be a (relatively) open neighbourhood of 0 in $\bar{\boldsymbol{S}}$ such that $c^* + \gamma$ and $c^{**} + \gamma$ lie in $\mathbb{R}^{\mathcal{S}}_+$ for all $\gamma \in \Omega$. Then, we let $\tilde{h}^*$: $\Omega \times \mathbb{R} \to \bar{\boldsymbol{S}}$ be defined as follows: for all $\gamma \in \Omega$ and all $\theta \in \mathbb{R}$

$$\tilde{h}^*(\gamma,\theta) = g(c^* + \gamma) + \theta\sum_{e\in\mathcal{E}} (c^*_e - (c^*_e + \gamma_e))e$$

That $\tilde{h}(\cdot,\cdot)$ does indeed take values in $\bar{\boldsymbol{S}}$ can be seen in the following way: Note that the sum following θ is the same as $P_{\mathcal{M}}(\gamma) - \gamma$. Recall that $P_{\mathcal{M}}(\cdot)$ takes values in $\Gamma_{\mathcal{M}}$, which in turn is contained in $\bar{\boldsymbol{S}}$. As each γ takes values in $\bar{\boldsymbol{S}}$, so then does $P_{\mathcal{M}}(\gamma) - \gamma$. Finally, recall that $g(\cdot)$ takes values in $\bar{\boldsymbol{S}}$.

By supposition

$$\tilde{h}^*(0,0) = g(c^*) = 0$$

Moreover, $d_\gamma\tilde{h}^*(0,0)\sigma = dg(c^*)\sigma$, $\forall\sigma \in \bar{\boldsymbol{S}}$. From this and (18), it follows that $d_\gamma\tilde{h}^*(0,0)$ is non-singular. From the implicit function theorem, then, there is a $\theta^* > 0$ such that, for all θ in the interval $(-\theta^*, \theta^*)$, there exists a $\gamma^*(\theta)$ satisfying (19), where $\tilde{c}^*(\theta) = c^* + \gamma^*(\theta)$.

$$\begin{aligned}0 = \tilde{h}^*(\gamma^*(\theta),\theta) &= g(c^* + \gamma^*(\theta)) + \theta\sum_{e\in\mathcal{E}}(c^*_e - (c^*_e + \gamma^*_e))e \\ &= \tilde{h}(\tilde{c}^*(\theta),\theta)\end{aligned} \tag{19}$$

Now, let $\tilde{h}^{**}$: $\Omega \times \mathbb{R} \to \bar{\boldsymbol{S}}$ be defined as follows: for all $\gamma \in \Omega$ and all $\theta \in \mathbb{R}$

$$\tilde{h}^{**}(\gamma,\theta) = g(c^{**} + \gamma) + \theta\sum_{e\in\mathcal{E}}(c^*_e - (c^{**}_e + \gamma_e))e$$

In this case, to see that $\tilde{h}^{**}(\cdot,\cdot)$ does indeed take values in $\bar{\boldsymbol{S}}$ is slightly more complicated. Note that

$$\sum_{e\in\mathcal{E}}(c^*_e - (c^{**}_e + \gamma_e))e = \sum_{e\in\mathcal{E}}(c^*_e - c^{**}_e)e - \sum_{e\in\mathcal{E}}\gamma_e e \tag{20}$$

As before, the second sum on the right of (20) is a member of $\bar{\boldsymbol{S}}$. On the other hand, the first term on the right is identical to $(c^* - c^{**}) - P_{\mathcal{M}}(c^* - c^{**})$. By supposition $c^* - c^{**}$ is a member of $\bar{\boldsymbol{S}}$. Moreover, $P_{\mathcal{M}}(\cdot)$ take values in $\Gamma_{\mathcal{M}}$, which is contained in $\bar{\boldsymbol{S}}$. All of this, taken with the fact that $g(\cdot)$ takes values in $\bar{\boldsymbol{S}}$, implies that $\tilde{h}^{**}(\cdot,\cdot)$ takes values in $\bar{\boldsymbol{S}}$.

Then we can argue, just as we argued for $\tilde{h}^*(\cdot,\cdot)$, that there is a $\theta^{**} > 0$ such that, for all θ in the interval $(-\theta^{**}, \theta^{**})$, there exists a $\gamma^{**}(\theta)$ satisfying

$$\begin{aligned}0 = \tilde{h}^{**}(\gamma^{**}(\theta),\theta) &= g(c^{**} + \gamma^{**}(\theta)) + \theta\sum_{e\in\mathcal{E}}(c^*_e - (c^{**}_e + \gamma^{**}_e(\theta)))e \\ &= \tilde{h}(\tilde{c}^{**}(\theta),\theta)\end{aligned} \tag{21}$$

where $\tilde{c}^{**}(\theta) = c^{**} + \gamma^{**}(\theta)$

By choosing $\theta^\dagger \in (0, \theta^*) \cap (0, \theta^{**})$ we obtain the desired result. □

6 Pathological example

Note that, when its hypothesis is satisfied, Theorem 2 does not entirely preclude multiple positive equilibria for the entrapped species differential equations; rather, it denies the possibility of two distinct non-degenerate positive equilibria. Our purpose here is to show that an entrapped species model (in fact, a mass-action model) can admit multiple degenerate positive equilibria even when the corresponding fully diffusive model cannot admit multiple positive equilibria of any kind. On the other hand, the example is hardly robust. Extremely slight perturbations of the example cause pathologies exhibited by it to vanish.

Consider the reaction network shown in (22).

$$\begin{aligned}&\mathrm{B}\leftarrow\mathrm{A}\rightarrow\mathrm{C}\\ &\mathrm{B}+\mathrm{C}\rightarrow 2\mathrm{A}\\ &\mathrm{D}\leftrightarrows\mathrm{E}\end{aligned} \tag{22}$$

We take the kinetics to be mass-action with every rate constant set to 1. With some effort it can be shown that, for this kinetic system, the fully diffusive model gives rise to precisely one positive equilibrium for all positive choices of $\bar{c}^0_{\mathrm{A}}, \ldots, \bar{c}^0_{\mathrm{E}}$ and $\bar{\alpha}_{\mathrm{A}}, \ldots, \bar{\alpha}_{\mathrm{E}}$.

Now, for the same kinetic system, consider an entrapped species model with $\mathcal{E} = \{\mathrm{A}, \mathrm{B}, \mathrm{C}\}$ and $\mathcal{M} = \{\mathrm{D}, \mathrm{E}\}$. The corresponding entrapped species model differential equations become those shown in (23). Moreover, it

$$\begin{aligned}\dot{c}_{\mathrm{A}} &= -2c_{\mathrm{A}} + 2c_{\mathrm{B}}c_{\mathrm{C}}\\ \dot{c}_{\mathrm{B}} &= c_{\mathrm{A}} - c_{\mathrm{B}}c_{\mathrm{C}}\\ \dot{c}_{\mathrm{C}} &= c_{\mathrm{A}} - c_{\mathrm{B}}c_{\mathrm{C}}\\ \dot{c}_{\mathrm{D}} &= c_{\mathrm{E}} - c_{\mathrm{D}} + \alpha_{\mathrm{D}}(c^0_{\mathrm{D}} - c_{\mathrm{D}})\\ \dot{c}_{\mathrm{E}} &= c_{\mathrm{D}} - c_{\mathrm{E}} + \alpha_{\mathrm{E}}(c^0_{\mathrm{E}} - c_{\mathrm{E}})\end{aligned} \tag{23}$$

Moreover, it is not hard to see that the equilibria of (23) consist of all compositions satisfying (24)–(26).

$$c_{\mathrm{A}} = c_{\mathrm{B}}c_{\mathrm{C}} \tag{24}$$

$$c_{\mathrm{D}} = c^{\mathrm{eq}}_{\mathrm{D}} := \frac{\alpha_{\mathrm{E}}c^0_{\mathrm{E}} + \alpha_{\mathrm{D}}c^0_{\mathrm{D}} + \alpha_{\mathrm{E}}\alpha_{\mathrm{D}}c^0_{\mathrm{D}}}{\alpha_{\mathrm{E}} + \alpha_{\mathrm{D}} + \alpha_{\mathrm{D}}\alpha_{\mathrm{E}}} \tag{25}$$

$$c_{\mathrm{E}} = c^{\mathrm{eq}}_{\mathrm{E}} := \frac{\alpha_{\mathrm{E}}c^0_{\mathrm{E}} + \alpha_{\mathrm{D}}c^0_{\mathrm{D}} + \alpha_{\mathrm{E}}\alpha_{\mathrm{D}}c^0_{\mathrm{E}}}{\alpha_{\mathrm{E}} + \alpha_{\mathrm{D}} + \alpha_{\mathrm{D}}\alpha_{\mathrm{E}}} \tag{26}$$

Our interest is in deciding whether there can be more than one positive equilibrium in the same entrapped species compatibility class. In this case, it is not hard to see that

$$\bar{\boldsymbol{S}} = \text{span}\{\mathrm{B}-\mathrm{A}, \mathrm{C}-\mathrm{A}, \mathrm{D}, \mathrm{E}\}$$

and that two compositions c and c' are entrapped species compatible if and only if $c_{\mathrm{A}} + c_{\mathrm{B}} + c_{\mathrm{C}} = c'_{\mathrm{A}} + c'_{\mathrm{B}} + c'_{\mathrm{C}}$.

In particular, we can study the equilibria residing in the entrapped species compatibilty class of compositions

characterised by the condition

$$c_A + c_B + c_C = 1 \tag{27}$$

From (24) to (27), it follows that there are an infinite number of equilibrium compositions within this compatibility class: these trace out a curve, parameterised by $c_B \in [0, 1]$, and given by

$$c_A = \frac{c_B(1 - c_B)}{1 + c_B}, \qquad c_C = \frac{1 - c_B}{1 + c_B}, \qquad c_D = c_D^{eq}, \qquad c_E = c_E^{eq} \tag{28}$$

It can be confirmed, however, that all such equilibria are degenerate. In fact, if c^* is a point along the curve given by (28), then any choice of a (non-zero) vector tangent to the curve at c^* is a member of ker $(dg(c^*))) \cap \bar{S}$.

The example itself is highly degenerate, for its capacity for multiple positive equilibria disappears completely when the reactions $A \to B$ and $A \to C$ are made very slightly reversible: in particular, consider network (29) and suppose again that the kinetics is mass-action with rate constants for reactions in the original network (22) set to 1 and with rate constants for the added reactions $B \to A$ and $C \to A$ both set to a very small number ε.

$$\begin{aligned} &B \rightleftarrows A \rightleftarrows C \\ &B + C \longrightarrow 2A \\ &D \rightleftarrows E \end{aligned} \tag{29}$$

In this case, the entrapped species model differential equations (corresponding to $\mathcal{E} = \{A, B, C\}$ and $\mathcal{M} = \{D, E\}$) become those shown in (30). It is not difficult to verify that, in contrast to the situation for $\varepsilon = 0$, the

$$\begin{aligned} \dot{c}_A &= -2c_A + 2c_B c_C + \varepsilon(c_B + c_C) \\ \dot{c}_B &= c_A - c_B c_C - \varepsilon c_B \\ \dot{c}_C &= c_A - c_B c_C - \varepsilon c_C \\ \dot{c}_D &= c_E - c_D + \alpha_D(c_D^0 - c_D) \\ \dot{c}_E &= c_D - c_E + \alpha_E(c_E^0 - c_E) \end{aligned} \tag{30}$$

entrapped species compatibility class corresponding to $c_A + c_B + c_C = 1$ now contains precisely one positive equilibrium c^*, no matter how small the rate constant $\varepsilon > 0$ might be. In fact, the equilibrium composition c^* is given by

$$c_A^* = (c_B^*) + \varepsilon c_B^*, \qquad c_B^* = \frac{-(2 + \varepsilon) + \sqrt{(2 + \varepsilon)^2 + 4}}{2}, \qquad c_C^* = c_B^*, \qquad c_D^* = c_D^{eq}, \qquad c_E^* = c_E^{eq}$$

where c_D^{eq} and c_E^{eq} are as in (25) and (26). We took the rate constants for the added reactions $B \to A$ and $C \to A$ to be equal only to indicate the pathology of the original example in a simple way. In fact, uniqueness of positive equilibria (in the sense of Section 3) for the entrapped species model obtains no matter what rate constants are assigned to the various reactions in network (29).

It is perhaps useful to summarise just how the example behaves: for network (22), taken with all mass-action rate constants set to 1, the fully diffusive model admits precisely one positive equilibrium for all values of the mass transfer coefficients and the ambient species concentrations. On the other hand, an entrapped species model (with $\mathcal{E} = \{A, B, C\}$ and $\mathcal{M} = \{D, E\}$) for the same kinetic system admits multiple positive equilibria (in fact, an infinite number) within the same entrapped species compatibility class. Nevertheless, these are all degenerate, and the behaviour is not robust: the capacity for multiple positive equilibria is destroyed by tiny perturbations of the underlying kinetic system.

Remark 1: That the elements of $\mathcal{E}$ and $\mathcal{M}$ appear in separate reactions is not consequential to the example, for other similar examples can be constructed in which elements of the two sets interact. The example used here was chosen for its simplicity, in particular so that its equilibria could be calculated easily. The structural origin of the pathology inherent in the example is discussed in Appendix IV of Feinberg [9]. (For mass-action kinetics, such pathologies do not arise when, for example, all reactions are reversible, however small some of the reverse rate constants might be.)

7 Concluding remarks

As we indicated in the Introduction, our interest is in extending results for fully diffusive models [1, 2] to entrapped species models. Theorem 2 does this to the extent that when the fully diffusive model for a given kinetic system does not have the capacity for multiple positive equilibria, then neither does any entrapped species model derived from the same kinetic system, except perhaps for degenerate multiple positive equilibria. (That is, if there are multiple positive equilibria, all but at most one are degenerate.)

It should be noted that Theorem 2 is written for arbitrary kinetic systems, not necessarily mass-action systems. The theorem as it stands is, for the most part, adequate for our purposes. We wish to point out, however, that for mass-action systems Theorem 2 lends itself to sharpening. In particular, it can be shown without much difficulty that for a reaction network that is injective (in the sense of Craciun and Feinberg [1]) the only possible way that multiple positive steady states might be exhibited is if all are degenerate. (That is, in contrast to the slightly weaker conclusion given by Theorem 2, not even one can be non-degenerate.) It should be kept in mind that Section 6 provides an example of a (structurally unstable) mass-action system for which an entrapped species model admits multiple (degenerate) steady states even when the fully diffusive model admits only a unique steady state. Nevertheless, it is possible to prove statements, restricted to certain broad classes of mass-action systems, that are sharper than Theorem 2 to the extent that the denial of multiple positive equilibria in the entrapped species model is total, unqualified by issues of degeneracy. We expect this to be the subject of a separate article.

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9 References

1 Craciun, G., and Feinberg, M.: 'Multiple equilibria in complex chemical reaction networks: I. The injectivity property', *SIAM J. Appl. Math.*, 2005, **65**, pp. 1526–1546
2 Craciun, G., and Feinberg, M.: 'Multiple equilibria in complex chemical reaction networks: II. The species-reaction graph', *SIAM J. Appl. Math.*, 2006, **66**, pp. 1321–1338
3 Lee, E., Salic, A., Kruger, R., Heinrich, R., and Kirschner, M.W.: 'The roles of APC and axin derived from experimental and theoretical analysis of the Wnt pathway', *PLoS Biol.*, 2003, **1**, pp. 116–132
4 Tyson, J.J., and Novak, B.: 'Modeling the control of DNA replication in fission yeast', *Proc. Nat. Acad. Sci. USA*, 1997, **94**, pp. 9147–9152

5 Wilson, R.: 'Introduction to Graph Theory' (Addison-Wesley, Boston, 1996)
6 Érdi, P., and Tóth, J.: 'Mathematical Models of Chemical Reactions: Theory and Applications of Deterministic and Stochastic Models' (Princeton University Press, Princeton, NJ, 1989)
7 Tóth, J.: 'Multistationarity is neither necessary nor sufficient for oscillations', *J. Math. Chem.*, 1999, **25**, pp. 393–397
8 Feinberg, M.: 'Lectures on Chemical Reaction Networks', 1979, available at http//www.che.eng.ohio-state.edu/~feinberg/LectureOnReactionNetworks
9 Feinberg, M.: 'Chemical reaction network structure and the stability of complex isothermal reactors – I. The deficiency zero and deficiency one theorems', *Chem. Eng. Sci.*, 1987, **42**, pp. 2229–2268