IDENTIFIABILITY OF CHEMICAL REACTION NETWORKS

Gheorghe Craciun[a,1]   Casian Pantea[b]

[1]**Corresponding Author.** *Address: University of Wisconsin-Madison, Department of Mathematics,*

480 Lincoln Drive, Madison WI 53706-1388

Phone:608-265-3391; Fax:608-263-8891; Email: `craciun@math.wisc.edu`

Key Words. Chemical reaction networks, Mass-action kinetics, Parameter identification

2000 Mathematics Subject Classification. Primary: 80A30; Secondary: 65H10, 37C25

ABSTRACT. We consider the dynamics of chemical reaction networks under the assumption of mass-action kinetics. We show that there exist reaction networks $\mathcal{R}$ for which the reaction rate constants are not uniquely identifiable, even if we are given complete information on the dynamics of concentrations for all chemical species of $\mathcal{R}$. Also, we show that there exist reaction networks $\mathcal{R}_1 \neq \mathcal{R}_2$ such that their dynamics are identical under appropriate choices of reaction rate constants, and present theorems that characterize the properties of $\mathcal{R}$, $\mathcal{R}_1$, $\mathcal{R}_2$ that make this possible. We use these facts to show how we can determine dynamical properties of some chemical networks by analyzing other chemical networks.

[a]Department of Mathematics and Department of Biomolecular Chemistry, University of Wisconsin, Madison, WI 53706.

[b]Department of Mathematics, University of Wisconsin, Madison, WI 53706.

1. INTRODUCTION

A chemical reaction network, under the assumption of mass-action kinetics, gives rise to a dynamical system governing the concentrations of the different chemical species [1–11]. We are interested in studying the inverse problem, i.e., the identifiability of the reaction network and of its reaction rate constants, given the dynamics of chemical species concentrations.

In modern chemical and biochemical research, it has become very common to collect detailed information on time-dependent chemical concentration data for large networks of chemical reactions (see survey papers [12, 13]). In most cases, only the identity of the chemical species present in the network is known, and the exact structure of the chemical reactions, as well as the reaction rate constants are unknown; in other cases the set of chemical reactions (i.e. the reaction network) is also known, and only the reaction rate constants are unknown.

A great variety of computational methods have been developed for the identification of chemical reaction networks and their reaction rate constants from time-dependent measurements of chemical species concentrations [14–21]. On the other hand, two different reaction networks might generate identical dynamical system models, making it impossible to discriminate between them, even if we are given experimental data of perfect accuracy and unlimited temporal resolution. (Sometimes this limitation is referred to as the "fundamental dogma of chemical kinetics", although it is actually not a well known fact in the engineering or biochemistry communities [13, 22, 23].) We approach this issue in section 4 where we describe necessary and sufficient conditions for two reaction networks to give rise to the same dynamical system model.

Also, we will show that, even if we know the reaction network that gives rise to the chemical dynamics under study, there might exist multiple sets of reaction rate constants that provide

perfect fit for the data since they give rise to identical dynamical system models. In section 3 we will describe necessary and sufficient conditions for the unique identifiability of the reaction rate constants of a chemical reaction network.

In section 5 we apply some of these results in the case of a reaction network with n species, that contains all possible reactions among all possible unimolecular and bimolecular complexes. This reaction network is very important in applications, since it is the default network used if no *a priori* information is provided on the reaction network structure [14]. We show that the number of unknown parameters (reaction rate constants) can be reduced from $O(n^4)$ to $O(n^3)$ without loss of generality on the kinetics, by considering dynamically equivalent reduced networks, but no reduced network with uniquely identifiable rate constants exists.

In section 6 we show how one can use these results to deduce qualitative information on the dynamics of reaction networks; in particular, we describe an example where we deduce that some reaction network cannot give rise to multiple positive equilibria for any values of its reaction rate constants.

2. Chemical reaction networks and mass-action kinetics

A chemical reaction network is usually given by a finite list of reactions that involve a finite set of chemical species. As an example, consider the reaction network with two species A_1 and A_2 schematically given in the diagram

$$(1) \qquad 2A_1 \rightleftharpoons A_1 + A_2 \rightleftharpoons 2A_2$$
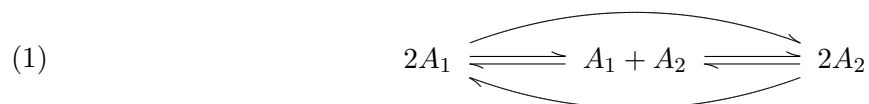
To keep track of the temporal evolution of the state of this chemical system, we define the functions $c_{A_1}(t)$ and $c_{A_2}(t)$ to be the molar concentrations of the species A_1, A_2 at time t. The chemical reactions in the network are responsible for changes in the concentrations; for instance, whenever the reaction $A_1 + A_2 \to 2A_1$ occurs, the net gain is a molecule of A_1, whereas one molecule of A_2 is lost. Similarly, the reaction $2A_2 \to 2A_1$ results in the the creation of two molecules of A_1 and the loss of two molecules of A_2.

To complete the setup, we need to quantify how often every reaction occurs. We will assume that the rate of change of the concentration of each species is governed by *mass-action kinetics* [1–11], i.e., that each reaction takes place at a rate that is proportional to the product of the concentrations of the species being consumed in that reaction. For example, under the mass-action kinetics assumption, the contribution of the reaction $A_1 + A_2 \to 2A_1$ to the rate of change of c_{A_1} has the form $k_{A_1+A_2\to 2A_1} c_{A_1} c_{A_2}$, where $k_{A_1+A_2\to 2A_1}$ is a positive number called *reaction rate constant*. In the same way, the reaction $2A_2 \to 2A_1$ contributes the negative value $-2k_{2A_2\to 2A_1} c_{A_2}^2$ to the rate of change of c_{A_2}. Collecting these contributions from all the reactions, we obtain the following system of differential equations associated to our chemical reaction network:

$$
\begin{aligned}
(2) \qquad \dot{c}_{A_1} = \ & -k_{2A_1\to A_1+A_2} c_{A_1}^2 + k_{A_1+A_2\to 2A_1} c_{A_1} c_{A_2} - k_{A_1+A_2\to 2A_2} c_{A_1} c_{A_2} \\
& + k_{2A_2\to A_1+A_2} c_{A_2}^2 - 2k_{2A_1\to 2A_2} c_{A_1}^2 + 2k_{2A_2\to 2A_1} c_{A_2}^2 \\
\dot{c}_{A_2} = \ & k_{2A_1\to A_1+A_2} c_{A_1}^2 - k_{A_1+A_2\to 2A_1} c_{A_1} c_{A_2} + k_{A_1+A_2\to 2A_2} c_{A_1} c_{A_2} \\
& - k_{2A_2\to A_1+A_2} c_{A_2}^2 + 2k_{2A_1\to 2A_2} c_{A_1}^2 - 2k_{2A_2\to 2A_1} c_{A_2}^2
\end{aligned}
$$

Definitions and notations. We now introduce the standard terminology of Chemical Reaction Network Theory (see [2, 4, 7]).

We denote by $\mathbb{R}$ the set of real numbers, by $\mathbb{R}_+$ the set of strictly positive real numbers, and by $\bar{\mathbb{R}}_+$ the set of nonnegative real numbers. For an arbitrary finite set I we denote by $\mathbb{R}^I$ the real vector space of all formal sums $\alpha = \sum_{i \in I} \alpha_i i$ for all $\alpha_i \in \mathbb{R}$. Similarly, we denote by $\mathbb{R}_+^I$ the set of formal sums $\alpha = \sum_{i \in I} \alpha_i i$ in which all α_i are strictly positive, and by $\bar{\mathbb{R}}_+^I$ the set of sums $\alpha = \sum_{i \in I} \alpha_i i$ in which all α_i are nonnegative. The *support* of an element $\alpha \in \mathbb{R}^I$ is $supp(\alpha) = \{i \in I : \alpha_i \neq 0\}$.

Definition 2.1. A *chemical reaction network* is a triple $(\mathscr{S}, \mathscr{C}, \mathscr{R})$, where $\mathscr{S}$ is the set of chemical *species*, $\mathscr{C} \subseteq \mathbb{R}_+^{\mathscr{S}}$ is the set of *complexes* (i.e., the objects on both sides of the reaction arrows), and $\mathscr{R}$ is a relation on $\mathscr{C}$, denoted $y \to y'$ and represents the set of *reactions* in the network. Moreover, the set $\mathscr{R}$ must satisfy the following three conditions: it cannot contain elements of the form $y \to y$; for any $y \in \mathscr{C}$ there exists some $y' \in \mathscr{C}$ such that either $y \to y'$ or $y' \to y$; and the union of the supports of all $y \in \mathscr{C}$ is $\mathscr{S}$.

In other words, the second condition above guarantees that each complex appears in at least one reaction, and the third condition says that each species appears in at least one complex. For the system (1), the set of species is $\mathscr{S} = \{A_1, A_2\}$, the set of complexes is $\mathscr{C} = \{2A_1, A_1 + A_2, 2A_2\}$ and the set of reactions is $\mathscr{R} = \{2A_1 \rightleftharpoons A_1 + A_2, A_1 + A_2 \rightleftharpoons 2A_2, 2A_2 \rightleftharpoons 2A_1\}$, and consists of 6 reactions, represented as three reversible reactions.

Note that we regard the complexes as formal linear combinations of the species; on the other hand, it will be useful to also think of the complexes as (column) vectors of dimension equal to the number of elements of $\mathscr{S}$, via an identification given by a fixed ordering of the species. For

example, the complexes above are $2A_1 = \begin{bmatrix} 2 \\ 0 \end{bmatrix}$, $A_1 + A_2 = \begin{bmatrix} 1 \\ 1 \end{bmatrix}$, and $2A_2 = \begin{bmatrix} 0 \\ 2 \end{bmatrix}$. *Among other*

advantages, this abuse of notation provides a convenient way of representing the reaction vectors

$y' - y$ *for all* $y \to y' \in \mathscr{R}$; *these vectors will play an important role in what follows.* Moreover,

for convenience, we will often refer to a chemical reaction network by specifying $\mathscr{R}$ only, since $\mathscr{R}$

encompasses all the information about the network.

Definition 2.2. A *mass-action system* is a quadruple $(\mathscr{S}, \mathscr{C}, \mathscr{R}, k)$, where $(\mathscr{S}, \mathscr{C}, \mathscr{R})$ is a chemical

reaction network and $k \in \mathbb{R}_+^{\mathscr{R}}$, where $k_{y \to y'}$ is the *reaction rate constant* of the reaction $y \to y' \in \mathscr{R}$.

In what follows, we will study mass-action systems only by looking at their structure, i.e. the

network $(\mathscr{S}, \mathscr{C}, \mathscr{R})$, thus deriving conclusions that are independent of the rate constants given by

the vector k.

Mass-action kinetics imposes dynamical constraints on a chemical system, represented by a well-

determined family of ordinary differential equations. Remarkably, using the notations described

above, this system of ODE's can be summarized in one compact equation. Before stating it we

need to introduce one more notation: given two vectors $u = \sum_{s \in \mathscr{S}} u_s s$ and $v = \sum_{s \in \mathscr{S}} v_s s$ in $\mathbb{R}_+^{\mathscr{S}}$, we

denote $u^v = \prod_{s \in \mathscr{S}} (u_s)^{v_s}$, with the convention $0^0 = 1$.

The system of differential equations for the mass-action chemical reaction network $(\mathscr{S}, \mathscr{C}, \mathscr{R}, k)$

is

$$(3) \qquad\qquad \dot{c} = \sum_{y \to y' \in \mathscr{R}} k_{y \to y'} c^y (y' - y),$$

where $c \in \mathbb{R}^{\mathscr{S}}$ is the positive vector of species concentrations.

The equation (3) is obtained in the same way we have obtained the system (2). The total rate of change is computed by summing the contributions of all the reactions in $\mathscr{R}$. Each reaction $y \to y'$ contributes proportionally to the product of the concentrations of the species in its *source* y, i.e., c^y, and also proportional to the number of molecules gained or lost in this reaction. Finally, the proportionality factor is $k_{y \to y'}$. For example, we can rewrite (2) in the vector form (3) as

$$(4) \quad \begin{bmatrix} \dot{c}_1 \\ \dot{c}_2 \end{bmatrix} = k_{2A_1 \to A_1+A_2} c^{2A_1} \begin{bmatrix} -1 \\ 1 \end{bmatrix} + k_{A_1+A_2 \to 2A_1} c^{A_1+A_2} \begin{bmatrix} 1 \\ -1 \end{bmatrix} + k_{A_1+A_2 \to 2A_2} c^{A_1+A_2} \begin{bmatrix} -1 \\ 1 \end{bmatrix}$$

$$+ k_{2A_2 \to A_1+A_2} c^{2A_2} \begin{bmatrix} 1 \\ -1 \end{bmatrix} + k_{2A_1 \to 2A_2} c^{2A_1} \begin{bmatrix} -2 \\ 2 \end{bmatrix} + k_{2A_2 \to 2A_1} c^{2A_2} \begin{bmatrix} 2 \\ -2 \end{bmatrix}.$$

The expression on the right hand side of equation (3) plays an important role in what follows. We denote it by $r(\mathscr{R}, k)$; in other words, we have:

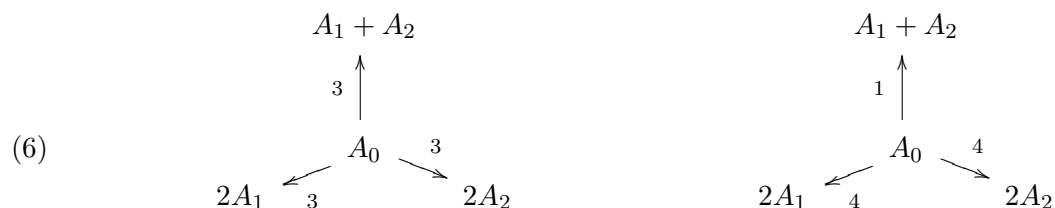$$(5) \qquad\qquad r(\mathscr{R}, k)(c) = \sum_{y \to y' \in \mathscr{R}} k_{y \to y'} c^y (y' - y)$$

for all $c \in \mathbb{R}_+^{\mathscr{S}}$.

3. IDENTIFIABILITY OF RATE CONSTANTS GIVEN A REACTION NETWORK AND ITS DYNAMICS

Suppose we are given a reaction network $(\mathscr{S}, \mathscr{C}, \mathscr{R})$ and, by fitting experimental data, we also know the differential equations (3) that govern the dynamics of the network. Then, we would like to determine the rate constants of the reactions, i.e., $k \in \mathbb{R}_+^{\mathscr{R}}$. We will see that, in general, the values of k's that fit the data are not unique. In other words, it might be impossible for us to

specify the "true" values of the rate constants, no matter how accurate the experimental data is, because there exist several different k's that give rise to exactly the same differential equations (3).

For example, note that for the reaction network in (6), both sets of rate constants shown below produce exactly the same dynamics, given by $\dot{c}_{A_0} = -9c_{A_0}$, $\dot{c}_{A_1} = \dot{c}_{A_2} = 9c_{A_0}$.

(6)

$$
\begin{array}{cc}
A_1 + A_2 & A_1 + A_2 \\
\uparrow \, 3 & \uparrow \, 1 \\
A_0 \,\,\, 3 & A_0 \,\,\, 4 \\
2A_1 \,\,\, 3 \quad 2A_2 & 2A_1 \,\,\, 4 \quad 2A_2
\end{array}
$$

Let us take a closer look at the mass-action systems in (6). Suppose the rate constants are unknown, but the differential equations

$$
\begin{aligned}
(7) \qquad \dot{c}_{A_0} &= -k_{A_0 \to 2A_1} c_{A_0} - k_{A_0 \to A_1 + A_2} c_{A_0} - k_{A_0 \to 2A_2} c_{A_0} &&= K_0 c_{A_0} \\
\dot{c}_{A_1} &= 2k_{A_0 \to 2A_1} c_{A_0} + k_{A_0 \to A_1 + A_2} c_{A_0} &&= K_1 c_{A_0} \\
\dot{c}_{A_2} &= k_{A_0 \to A_1 + A_2} c_{A_0} + 2k_{A_0 \to 2A_2} c_{A_0} &&= K_2 c_{A_0}
\end{aligned}
$$

are known, i.e., K_0, K_1 and K_2 are known. The rate constants are then solutions of the (rank 2) linear system of equations

$$(8) \quad -k_{A_0 \to 2A_1} - k_{A_0 \to A_1 + A_2} \quad -k_{A_0 \to 2A_2} \quad = K_0$$

$$2k_{A_0 \to 2A_1} + k_{A_0 \to A_1 + A_2} \quad\quad\quad\quad = K_1$$

$$k_{A_0 \to A_1 + A_2} + 2k_{A_0 \to 2A_2} \quad = K_2$$

which does not have unique solution. (On the other hand, we assume that at least one solution exists.)

The obstacle that prevents identifiability is therefore the linear dependence of the vectors $[-1, 2, 0]^t$, $[-1, 1, 1]^t$ and $[-1, 0, 2]^t$, i.e., precisely the three vectors of the form $y' - y$, where $y = A_0$ and $y \to y'$ is a reaction (here v^t denotes the transpose of v). Should our reaction network had been $2A_1 \leftarrow A_0 \to 2A_2$, the system (8) would have had a unique solution, and the rate constants would have been determined uniquely.

Definition 3.1. We say that a reaction network $(\mathscr{S}, \mathscr{C}, \mathscr{R})$ *has uniquely identifiable rate constants if $r(\mathscr{R}, k') \neq r(\mathscr{R}, k'')$ for any distinct rate constant vectors k', $k'' \in \mathbb{R}_+^{\mathscr{R}}$.*

Then we have:

Theorem 3.2. *Under the mass-action kinetics assumption, a reaction network $(\mathscr{S}, \mathscr{C}, \mathscr{R})$ has uniquely identifiable rate constants if and only if for each source complex $y_0 \in \mathscr{C}$, the reaction vectors $\{y' - y_0 : y_0 \to y' \in \mathscr{R}\}$ are linearly independent.*

Proof. Suppose the reaction vectors $\{y' - y : y \to y' \in \mathscr{R}\}$ are linearly independent for each source complex y and $r(\mathscr{R}, k') = r(\mathscr{R}, k'')$ for some rate constants vectors k', k''. We therefore have

$$\sum_{y \to y' \in \mathscr{R}} k'_{y \to y'} c^y (y' - y) = \sum_{y \to y' \in \mathscr{R}} k''_{y \to y'} c^y (y' - y)$$

for all $c \in \mathbb{R}_+^{\mathscr{S}}$, or

$$(9) \qquad \sum_{y \in \mathscr{C}} \Big(\sum_{\{y' : y \to y' \in \mathscr{R}\}} (k'_{y \to y'} - k''_{y \to y'})(y' - y) \Big) c^y = 0$$

for all $c \in \mathbb{R}_+^S$. The vector components of the left hand side of (9) are polynomial functions in c, identically equal to zero on the positive orthant; therefore they must have zero coefficients. This implies that

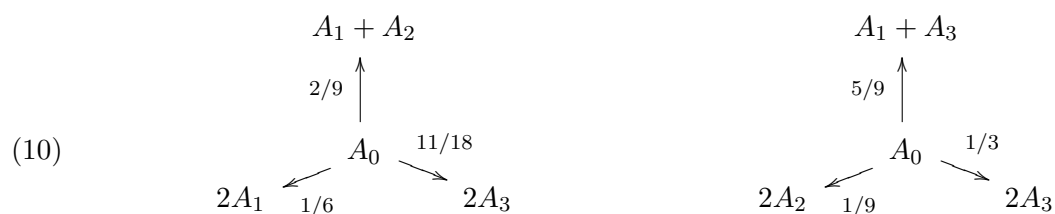$$\sum_{\{y' : y \to y' \in \mathscr{R}\}} (k'_{y \to y'} - k''_{y \to y'})(y' - y) = 0$$

for all source complexes $y \in \mathscr{C}$. Since, for each source complex $y \in \mathscr{C}$ the vectors $\{y' - y : y \to y' \in \mathscr{R}\}$ are linearly independent, we conclude that $k'_{y \to y'} = k''_{y \to y'}$ for all reactions $y \to y'$ in $\mathscr{R}$.

Conversely, suppose for some source complex y_0 the vectors $\{y' - y_0 : y_0 \to y' \in \mathscr{R}\}$ are linearly dependent. Then $\sum_{y_0 \to y' \in \mathscr{R}} \alpha_{y_0 \to y'}(y' - y_0) = 0$ for some real numbers $\alpha_{y_0 \to y'}$, not all zero. We can choose k', $k'' \in \mathbb{R}_+^{\mathscr{R}}$ such that $k'_{y_0 \to y'} - k''_{y_0 \to y'} = \alpha_{y_0 \to y'}$ for all reactions $y_0 \to y' \in \mathscr{R}$ and $k'_{y \to y'} = k''_{y \to y'}$ for all reactions $y \to y' \in \mathscr{R}$ with source $y \neq y_0$. Then the two rate constant vectors k' and k'' are distinct, but equation (9) shows that $r(\mathscr{R}, k') = r(\mathscr{R}, k'')$. $\qquad \square$

Remark. Note that if there are $k', k'' \in \mathbb{R}_+^{\mathscr{R}}$ such that $r(\mathscr{R}, k') = r(\mathscr{R}, k'')$, then this implies that *for any* $k \in \mathbb{R}_+^{\mathscr{R}}$ there exists $\tilde{k} \in \mathbb{R}_+^{\mathscr{R}}$ such that $r(\mathscr{R}, k) = r(\mathscr{R}, \tilde{k})$.

4. IDENTIFIABILITY OF THE REACTION NETWORK GIVEN THE DYNAMICS

In the preceding section we have shown that, given a reaction network and the differential equations that determine its dynamics, it might be impossible to identify its rate constants uniquely. Now we will argue that, given the dynamics, it might be impossible to identify the reaction network uniquely (see [22, page 66]). For instance, let us consider the following example:

$$
\begin{array}{cc}
A_1 + A_2 & A_1 + A_3 \\
\uparrow{\scriptstyle 2/9} & \uparrow{\scriptstyle 5/9} \\
A_0 \;\xrightarrow{11/18} & A_0 \;\xrightarrow{1/3} \\
2A_1 \;{\scriptstyle 1/6}\qquad 2A_3 & 2A_2 \;{\scriptstyle 1/9}\qquad 2A_3
\end{array}
$$

(10)

Although they are distinct, these two reaction networks give rise to the same differential equations:

(11)
$$\dot{c}_{A_0} = (-\frac{2}{9} - \frac{1}{6} - \frac{11}{18})c_{A_0} = - c_{A_0} \qquad\qquad \dot{c}_{A_0} = (-\frac{5}{9} - \frac{1}{9} - \frac{1}{3})c_{A_0} = -c_{A_0}$$

$$\dot{c}_{A_1} = (\frac{2}{9} + 2(\frac{1}{6}))c_{A_0} \quad = \frac{5}{9}c_{A_0} \qquad\qquad \dot{c}_{A_1} = \qquad\qquad \frac{5}{9}c_{A_0} = \frac{5}{9}c_{A_0}$$

$$\dot{c}_{A_2} = \frac{2}{9}c_{A_0} \qquad\qquad = \frac{2}{9}c_{A_0} \qquad\qquad \dot{c}_{A_2} = \qquad\quad 2(\frac{1}{9})c_{A_0} = \frac{2}{9}c_{A_0}$$

$$\dot{c}_{A_3} = 2(\frac{11}{18})c_{A_0} \qquad\quad = \frac{11}{9}c_{A_0} \qquad\qquad \dot{c}_{A_3} = \quad (\frac{5}{9} + 2(\frac{1}{3}))c_{A_0} = \frac{11}{9}c_{A_0}$$

We conclude that, in this case, no matter how accurately we fit our experimental data, we cannot determine uniquely the chemical reaction network that generates the observed dynamics, because there exist multiple networks that produce exactly the same differential equations.

In the light of this fact, it is natural to ask: when can two different reaction networks generate the same differential equations? Whenever this is true, we will call these two reaction networks *confoundable*. An answer to this question is given in Theorem 4.4.

Example (10) illustrates well what happens in general. From equations (11) we see that the two networks shown in (10) produce the same dynamics because the vector $[-1, 5/9, 2/9, 11/9]$ can be written as

$$
\begin{bmatrix} -1 & -1 & -1 \\ 1 & 2 & 0 \\ 1 & 0 & 0 \\ 0 & 0 & 2 \end{bmatrix} \begin{bmatrix} 2/9 \\ 1/6 \\ 11/8 \end{bmatrix},
$$

and as

$$
\begin{bmatrix} -1 & -1 & -1 \\ 1 & 0 & 0 \\ 0 & 2 & 0 \\ 1 & 0 & 2 \end{bmatrix} \begin{bmatrix} 5/9 \\ 1/9 \\ 1/3 \end{bmatrix}.
$$

Denote $K = [0, 5/9, 2/9, 11/9]$. Then

$$
[-1, 5/9, 2/9, 11/9] = K - A_0 = \frac{2}{9}(A_1 + A_2 - A_0) + \frac{1}{6}(2A_1 - A_0) + \frac{11}{8}(2A_3 - A_0) =
$$

$$
= \frac{5}{9}(A_1 + A_3 - A_0) + \frac{1}{9}(2A_2 - A_0) + \frac{1}{3}(2A_3 - A_0).
$$

Therefore, confoundability is due to the nonempty intersection of the convex cones generated by the reaction vectors in the two networks; this is illustrated in Figure 1.

In order to formulate Theorem 4.4 we need the following definitions:

Definition 4.1. Let $(\mathscr{S}, \mathscr{C}, \mathscr{R})$ be a chemical reaction network. Define the following family of functions:

$$Dyn(\mathscr{R}) = \left\{ r(\mathscr{R}, k) : k \in \mathbb{R}_+^{\mathscr{R}} \right\}.$$

Recall that, as defined in (5), $r(\mathscr{R}, k)(c) = \displaystyle\sum_{y \to y' \in \mathscr{R}} k_{y \to y'} c^y (y' - y)$ for all concentration vectors $c \in \mathbb{R}_+^{\mathscr{S}}$.

Definition 4.2. Two chemical reaction networks $(\mathscr{S}, \mathscr{C}', \mathscr{R}')$ and $(\mathscr{S}, \mathscr{C}'', \mathscr{R}'')$ are called *confoundable* if $Dyn(\mathscr{R}') \cap Dyn(\mathscr{R}'') \neq \emptyset$.

In other words, two reaction networks are confoundable if they produce the same mass-action differential equations for some choice of the rate constants. In that case, we cannot distinguish between the two reaction networks by fitting experimental data. Note that the source complexes $y \in \mathscr{C}$ appear as exponents of c in the polynomials of $Dyn(\mathscr{R})$, and no other complexes have this property; therefore, $Dyn(\mathscr{R})$ specifies them uniquely. This implies that, if two chemical reaction systems are confoundable, then their source complexes must be the same.

Motivated by the discussion of the example (10), we introduce the following definition.

Definition 4.3. For a reaction network $(\mathscr{S}, \mathscr{C}, \mathscr{R})$ and $y_0 \in \mathscr{C}$ we denote by

$$Cone_{\mathscr{R}}(y_0) = \left\{ \sum_{y_0 \to y' \in \mathscr{R}} \alpha_{y_0 \to y'}(y' - y_0) : \ \alpha_{y_0 \to y'} > 0 \text{ for all } y_0 \to y' \in \mathscr{R} \right\},$$

the open convex cone generated by the set $\{y' - y_0 : y_0 \to y' \in \mathscr{R}\}$.

(For more information on convex cones and their generating sets see [24].) Then we have:

Theorem 4.4. *Under the mass-action kinetics assumption, two chemical reaction networks* $(\mathscr{S}, \mathscr{C}', \mathscr{R}')$ *and* $(\mathscr{S}, \mathscr{C}'', \mathscr{R}'')$ *are confoundable if and only if they have the same source complexes and* $Cone_{\mathscr{R}'}(y) \cap Cone_{\mathscr{R}''}(y)$ *is nonempty for every source complex y.*

Proof. $Dyn(\mathscr{R}') \cap Dyn(\mathscr{R}'') \neq \emptyset$ if and only if there are rate constant vectors k' and k'' for $\mathscr{R}'$ and $\mathscr{R}''$ respectively, so that $\displaystyle\sum_{y \to y_1' \in \mathscr{R}'} k'_{y \to y_1'} c^y (y_1' - y) = \sum_{y \to y_2' \in \mathscr{R}''} k''_{y \to y_2'} c^y (y_2' - y)$ for all $c \in \mathbb{R}_+^S$. We rewrite this as

$$(12) \qquad \sum_{y \in \mathscr{C}} \Big(\sum_{\{y_1' : y \to y_1' \in \mathscr{R}'\}} k'_{y \to y_1'} (y_1' - y) - \sum_{\{y_2' : y \to y_2' \in \mathscr{R}''\}} k''_{y \to y_2'} (y_2' - y) \Big) c^y = 0$$

for all $c \in \mathbb{R}_+^S$. The vector components of the left hand side of (12) are polynomial functions in c, identically zero on the positive orthant, therefore having zero coefficients. Then (12) is equivalent to $\displaystyle\sum_{\{y_1' : y \to y_1' \in \mathscr{R}'\}} k'_{y \to y_1'} (y_1' - y) - \sum_{\{y_2' : y \to y_2' \in \mathscr{R}''\}} k''_{y \to y_2'} (y_2' - y) = 0$ for each source complex y, i.e., $Cone_{\mathscr{R}'}(y) \cap Cone_{\mathscr{R}''}(y) \neq \emptyset$ for all source complexes y. $\qquad\square$

Theorem 4.4 gives a necessary and sufficient condition for two reaction networks $\mathscr{R}'$, $\mathscr{R}''$ to be capable of producing the same dynamics for some choice of their two sets of rate constants. Note that the proof of the theorem actually says more: the two reaction networks are *unconditionally confoundable*, i.e., $Dyn(\mathscr{R}) = Dyn(\mathscr{R}'')$, if and only if $Cone_{\mathscr{R}'}(y) = Cone_{\mathscr{R}''}(y)$ for all source complexes y. If two reaction networks are unconditionally confoundable, then, no matter what the rate constants of one network are, there is a set of rate constants for the other one that give the same dynamics. This allows the reduction of the number of reactions in a network, without loss of generality of its dynamics, by considering only reactions that correspond to a set of generators of each cone $Cone_{\mathscr{R}}(y)$. This fact will be illustrated by an example in the next section.

5. APPLICATIONS: MODELING UNIMOLECULAR AND BIMOLECULAR REACTION NETWORKS

Suppose we are given experimental results for known chemical species $A_1, \ldots, A_n$, but we don't know what the reactions are; suppose, however, that all complexes are of the form A_i, $2A_i$ or $A_i + A_j$. Since we are able to observe the dynamics, it is natural to try to construct a corresponding reaction network model that takes into account all the possible reactions, even if for some reactions the rate constants will turn out very small or zero. However, since we know from section 4 that there might be multiple reaction networks that produce the same dynamics, we might as well look for a model that has a minimal number of reactions. This is helpful from a practical point of view since it significantly reduces the number of parameters (i.e., reaction rate constants) involved.

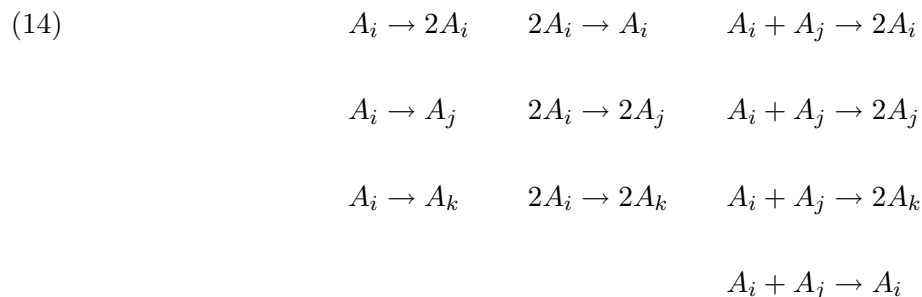For example, consider the case of 3 species, A_1, A_2, A_3. The "full" network is composed of reactions

$$(13) \qquad \{A_i \to A_j\}_{i \neq j}, \ A_i \to A_j + A_k, \ A_i + A_j \to A_k, \ \{A_i + A_j \to A_k + A_l\}_{\{i,j\} \neq \{k,l\}},$$

where $i, j, k \in \{1, 2, 3\}$. In other words, we are allowing all reactions involving unimolecular and bimolecular complexes, except for the trivial reactions $y \to y$. (Note that reactions that do not conserve mass, such as $A_i \to A_i + A_j$, may account for the existence of chemical species that are present in great excess, and whose dynamics can be neglected since their concentrations are practically constant; in other words, these reactions could be understood as $A_i + X \to A_i + A_j$, where the concentration of X is practically constant. See [2,7] for more details.)

Let us count the reactions in this model. Since we allow all the possible reactions between the unimolecular or bimolecular complexes, except for $y \to y$, any complex is a source in $N-1$ reactions, where N is the number of complexes. Therefore the total number of reactions is $N(N-1)$. There are 3 unimolecular complexes; for the bimolecular complexes $A_i + A_j$ there are two cases, according

to whether i is equal or different from j, and we count $\binom{3}{2} = 3$ complexes $A_i + A_j$ with $i \neq j$ and

3 complexes $2A_i$. Therefore $N = 9$ and the full network (13) has $9 \cdot 8 = 72$ reactions.

On the other hand, a "reduced" network that covers all the possible dynamical systems produced

by the full network can be obtained by using only the reactions corresponding to generating rays of

each cone $Cone_{\mathscr{R}}(y)$, for all sources y, as explained at the end of section 4. Diagrams corresponding

to each of the three different types of sources are given in the Figure 2. The lines joining the source

complex with all other complexes represent the reactions; the thick lines are extreme rays of the

corresponding cone, and therefore they give the reduced reaction network

$$(14) \qquad \begin{array}{lll} A_i \to 2A_i & 2A_i \to A_i & A_i + A_j \to 2A_i \\[2mm] A_i \to A_j & 2A_i \to 2A_j & A_i + A_j \to 2A_j \\[2mm] A_i \to A_k & 2A_i \to 2A_k & A_i + A_j \to 2A_k \\[2mm] & & A_i + A_j \to A_i \end{array}$$

where (i, j, k) are a permutation of $(1, 2, 3)$ and the expression $A_i + A_j$ assumes $i < j$. Note that

this choice of reduced network is not unique: except for the case of unimolecular source y we can

choose different generators for $Cone_{\mathscr{R}}(y)$. For example, the reaction $A_i + A_j \to A_i$ can be replaced

by $A_i + A_j \to A_j$, the reaction $A_i + A_j \to 2A_k$ can be replaced by $A_i + A_j \to A_i + A_k$ and $2A_i \to 2A_j$

can be replaced by $2A_i \to A_i + A_j$.

In the case shown in Figure 2(a) the minimal set of reactions (i.e., reactions whose vectors

generate $Cone_{\mathscr{R}}(A_1)$) is unique. Moreover, Theorem 3.1 guarantees that the rate constants of these

reactions are uniquely determined, since these three vectors are linearly independent. In the case shown in Figure 2(b) the minimal set of reactions is *not* unique; however, given any minimal set of reactions, the corresponding rate constants are uniquely determined, by Theorem 3.1. Interestingly, in the case shown in figure 2(c), the choice of the minimal set of reactions is not unique, *and* no matter which minimal set of reactions is chosen, the rate constants are *not* uniquely determined, also by Theorem 3.1. In other words: *no dynamically equivalent subnetwork has uniquely identifiable rate constants in this case. This fact must be taken into account whenever we try to design a numerical procedure to estimate the rate constant vector k from experimental data.*

Even if the minimal set of reactions is not unique, its cardinality is always the same. Specifically, in our case it contains 3(3+3+4)=30 reactions, which is a significant reduction from 72 reactions in the full network.

A version of the computation above works in general, in the case of n species. There are $(n + \frac{n(n-1)}{2} + n)$ complexes and therefore there are $(2n + \frac{n(n-1)}{2})(2n + \frac{n(n-1)}{2} - 1)$ reactions. A reduced model can be constructed by generalizing the reaction network (14). Namely, for source complexes A_i we choose the reactions $A_i \to 2A_i$ and $A_i \to A_j$ for all $j \neq i$; for source complexes $2A_i$, we choose the reactions $2A_i \to A_i$, $2A_i \to 2A_j$, for all $i \neq j$; and for source complexes $A_i + A_j$ with $i < j$ we choose the reactions $A_i + A_j \to 2A_k$ for all $k \in \{1, \dots, n\}$, and also the reaction $A_i + A_j \to A_i$. To check that these three sets of reactions do indeed generate the corresponding cones, we describe a simple algebraic computation. For example, for the unimolecular source A_1 we need to check that the n reaction vectors in the reduced network

$$A_1 = \begin{bmatrix} 1 \\ 0 \\ 0 \\ 0 \\ \vdots \\ 0 \end{bmatrix}, A_2 - A_1 = \begin{bmatrix} -1 \\ 1 \\ 0 \\ 0 \\ \vdots \\ 0 \end{bmatrix}, A_3 - A_1 = \begin{bmatrix} -1 \\ 0 \\ 1 \\ 0 \\ \vdots \\ 0 \end{bmatrix}, \ldots, A_n - A_1 = \begin{bmatrix} -1 \\ 0 \\ 0 \\ \vdots \\ 0 \\ 1 \end{bmatrix},$$

conically generate all the other reaction vectors with source A_1, namely,

$$(15) \qquad \begin{bmatrix} -1 \\ * \\ * \\ 2 \\ * \\ * \end{bmatrix}, \begin{bmatrix} -1 \\ * \\ 1 \\ * \\ 1 \\ * \end{bmatrix} \text{ and } \begin{bmatrix} 0 \\ * \\ * \\ 1 \\ * \\ * \end{bmatrix}$$

where "*" represent vertical sequences of zeros, possibly empty. The first vector corresponds to reactions $A_1 \rightarrow 2A_i$, $i \neq 1$, the second to $A_1 \rightarrow A_i + A_j$, where $i \neq j$ are different from 1 and the third one represents reactions $A_1 \rightarrow A_1 + A_i$, $i \neq 1$. It is easy to see that this is indeed true: for example, the third vector in (15) is $A_i = [0, \ldots, 1, \ldots, 0]^t = A_1 + A_i - A_1$ so it belongs to $Cone_{\mathscr{R}}(A_1)$ for any i; then $A_i + A_i - A_1$ and $A_i + A_j - A_1$ (the first and the second vectors in (15)) are also in $Cone_{\mathscr{R}}(A_1)$.

The number of reactions in the reduced network is $n^2 + n^2 + \frac{n(n-1)}{2}(n + 1) = 2n^2 + \frac{n(n^2-1)}{2}$; therefore, we reduced the number of reactions under consideration from $O(n^4)$ to $O(n^3)$.

6. Transfer of properties: an SR Graph example

Some of the results discussed in section 4 can be used to deduce dynamical properties of a reaction network by studying another network. More precisely, if two reaction networks $\mathscr{R}'$, $\mathscr{R}''$ have the property that $Dyn(\mathscr{R}') \subseteq Dyn(\mathscr{R}'')$ then dynamical properties of $\mathscr{R}'$ can be inferred from looking at the (possibly simpler) network $\mathscr{R}''$. Such properties are therefore *transferred* from $\mathscr{R}''$ to $\mathscr{R}'$.

We illustrate these considerations in what follows. Denote by $\mathscr{R}'$ the reaction network shown on the left side of (16) and by $\mathscr{R}''$ the reaction network shown on the right side of (16).

(16)
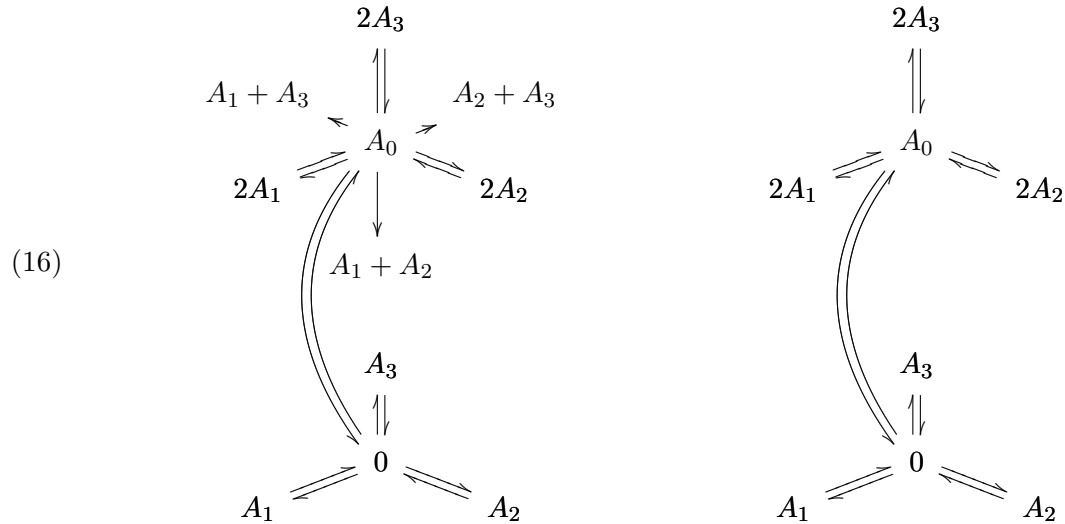
In this example, in addition to regular reactions, we are allowing inflow and outflow for all the species, and we model this in terms of special "reactions" $0 \to A_i$ and $A_i \to 0$ (see [7] for a detailed discussion of inflow and outflow reactions in the context of mass-action kinetics).

It is easy to see that $Cone_{\mathscr{R}'}(0) = Cone_{\mathscr{R}''}(0)$, $Cone_{\mathscr{R}'}(A_i) = Cone_{\mathscr{R}''}(A_i)$ for all $i \in \{0, 1, 2, 3\}$, and $Cone_{\mathscr{R}'}(2A_i) = Cone_{\mathscr{R}''}(2A_i)$ for all $i \in \{1, 2, 3\}$; therefore $Dyn(\mathscr{R}') = Dyn(\mathscr{R}'')$. We will show

that $\mathscr{R}'$ cannot have multiple equilibria for any value of its rate constants, by showing that $\mathscr{R}''$ has this property. Our tool will be the SR graph theorem, whose hypotheses are satisfied by $\mathscr{R}''$ but not by $\mathscr{R}'$. Therefore, this reduction allows us to extend the applicability of the SR graph Theorem.

The SR (Species-Reactions) graph is a bipartite graph introduced in [8]; its set of *nodes* consists of species and reactions (each reaction or pair of reversible reactions appear in a single node). The *edges* of the graph connect species nodes and reaction nodes as follows: if a species appears in a reaction, then there is an edge joining the corresponding species and reaction nodes; moreover, that edge is *labeled* with the complex in which the species appears. The two SR graphs associated to the reaction networks $\mathscr{R}'$ and $\mathscr{R}''$ are depicted in Figures 3 and 4, respectively. (See [6] for more examples.)

A little more terminology is needed in preparation for the SR graph result. A *c-pair* (complex pair) is a pair of edges that meet at a reaction node and have the same label. A cycle of the SR graph that contains an odd number of c-pairs is called an *o-cycle* (odd cycle), whereas cycles that contain an even number of c-pairs are called *e-cycles* (even cycles). In particular, cycles with no c-pairs are e-cycles. The *stoichiometric coefficient* of an edge is the coefficient of the adjacent species in the complex label of the edge. Cycles for which alternately multiplying and dividing the stoichiometric coefficients along its edges gives the result 1 are called *s-cycles*. We say that two cycles *split a c-pair* if there is a c-pair that lies in the union of the sets of edges of the two cycles, but not in their intersection.

The SR graph is used in [8] to discriminate between chemical reaction networks that can admit multiple equilibria and those that cannot. Our present purpose is to determine the capacity for multiple equilibria of the reaction network $\mathscr{R}'$, and we will use the following result ([8, Corollary 7.2]):

Theorem 6.1. *Let $\mathscr{R}$ be a reaction network such that all the cycles of its SR graph are o-cycles or s-cycles, and no two e-cycles split a c-pair. Then the mass-action dynamical system associated to $\mathscr{R}$ cannot have multiple positive equilibria, for any value of the rate constant vector k.*

The SR graph of $\mathscr{R}'$ in Figure 3 fails to satisfy the hypothesis of this theorem. For example, the cycle that goes through the nodes A_0, $A_0 \rightleftharpoons 2A_1$, A_1 and $A_0 \to A_1 + A_2$ does not contain any c-pairs, so it is not an o-cycle, but it is also not an s-cycle: the stoichiometric coefficients of its edges are 1, 2, 1, 1 and the result of the alternate multiplication and division is 2 or 1/2, depending on which edge one starts. Therefore Theorem 6.1 does not apply, and we are not able to draw an immediate conclusion on the existence of multiple equilibria for $\mathscr{R}'$. However, the hypotheses of the theorem are trivially satisfied by the SR graph of $\mathscr{R}''$ which has no cycles. We conclude that $\mathscr{R}''$ cannot have multiple equilibria and extend this conclusion to $\mathscr{R}'$, because $Dyn(\mathscr{R}') = Dyn(\mathscr{R}'')$.

References

[1] M. Feinberg, in: *Chemical Reactor Theory: A Review*, eds. N. Amundson and L. Lapidus, (Prentice-Hall, Englewood Cliffs, NJ, 1977), pp. 1–78.

[2] M. Feinberg, *Lectures on Chemical Reaction Networks*, written version of lectures given at the Mathematical Research Center, University of Wisconsin, Madison, WI, 1979. Available online from www.chbmeng.ohio-state.edu/~feinberg/LecturesOnReactionNetworks.

[3] M. Feinberg, in: *Patterns and Dynamics in Reactive Media*, eds. R. Aris, D. G. Aronson, and H. L. Swinney, IMA Vol. Math. Appl., (Springer, Berlin, 37, 1991), pp. 43–70.

[4] M. Feinberg, Arch. Rational Mech. Anal. 132 (1995) 311.

[5] M. Feinberg, Arch. Rational Mech. Anal. 132 (1995) 371.

[6] G. Craciun, Y. Tang, and M. Feinberg, Proceedings of the National Academy of Sciences 103:23 (2006) 8697.

[7] G. Craciun and M. Feinberg, SIAM J. Appl . Math. 65 (2005) 1526.

[8] G. Craciun and M. Feinberg, SIAM J. Appl . Math. 66 (2006) 1321.

[9] G. Craciun and M. Feinberg, IEE Proceedings Systems Biology 153:4 (2006) 179.

[10] B. D. Aguda, G. Craciun and R. Cetin-Atalay, in: *Data Sources and Computational Approaches for Generating Models of Gene Regulatory Networks, Reviews in Computational Chemistry, Vol 21*, eds. Kenny Lipkowitz, Raima Larter, and Thomas R. Cundari, (2005).

[11] E. Sontag, IEEE Trans. Automat. Control 46 (2001) 1028.

[12] G. Maria, Chem. Biochem. Eng. Q. 18:3 (2004) 195.

[13] E.J. Crampin, S. Schnell, and P. E. McSharry, Prog. Biophys. Mol. Biol. 86 (2004) 177.

[14] A. Karnaukhov, E. Karnaukhova and J. Williamson, Biophys. J. 92 (2007) 3459.

[15] S. Vajda, P. Valko and A. Yermakova, Computers and Chemical Engineering 10 (1986) 49.

[16] L.H. Hosten, Computers and Chemical Engineering 3 (1979) 117.

[17] D.M. Himmelau, C.R. Jones and K.B. Bischoff, Ind. Eng. Chem. Fundam. 6:4 (1967) 539.

[18] S. Schuster, C. Hilgetag, J.H. Woods and D.A. Fell, J. Math. Biol. 45 (2002) 153.

[19] E. Rudakov, Kinetics and Catalysis 1 (1960) 177.

[20] E. Rudakov, Kinetics and Catalysis 11 (1970) 228.

[21] L. Fay and A. Balogh, Acta Chim. Acad. Sci. Hun. 57:4 (1968) 391.

[22] P. Erdi and J. Toth, *Mathematical Models of Chemical Reactions: Theory and Applications of Deterministic and Stochastic Models*, (Princeton University Press, 1989)

[23] I.R Epstein and J.A. Pojman, *An Introduction to Nonlinear Chemical Dynamics: Oscillations, Waves, Patterns, and Chaos*, (Oxford University Press, 2002)

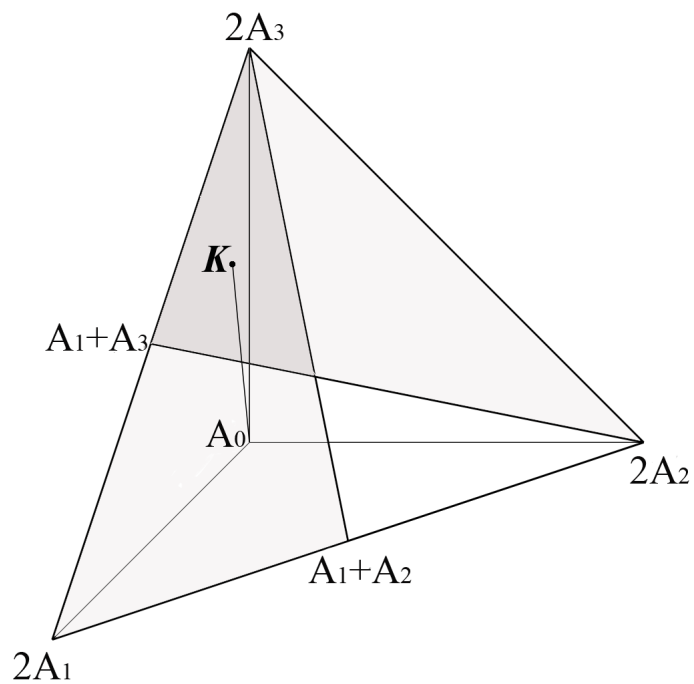[24] R.T. Rockafellar, *Convex Analysis*, (Princeton, NJ, 1970).

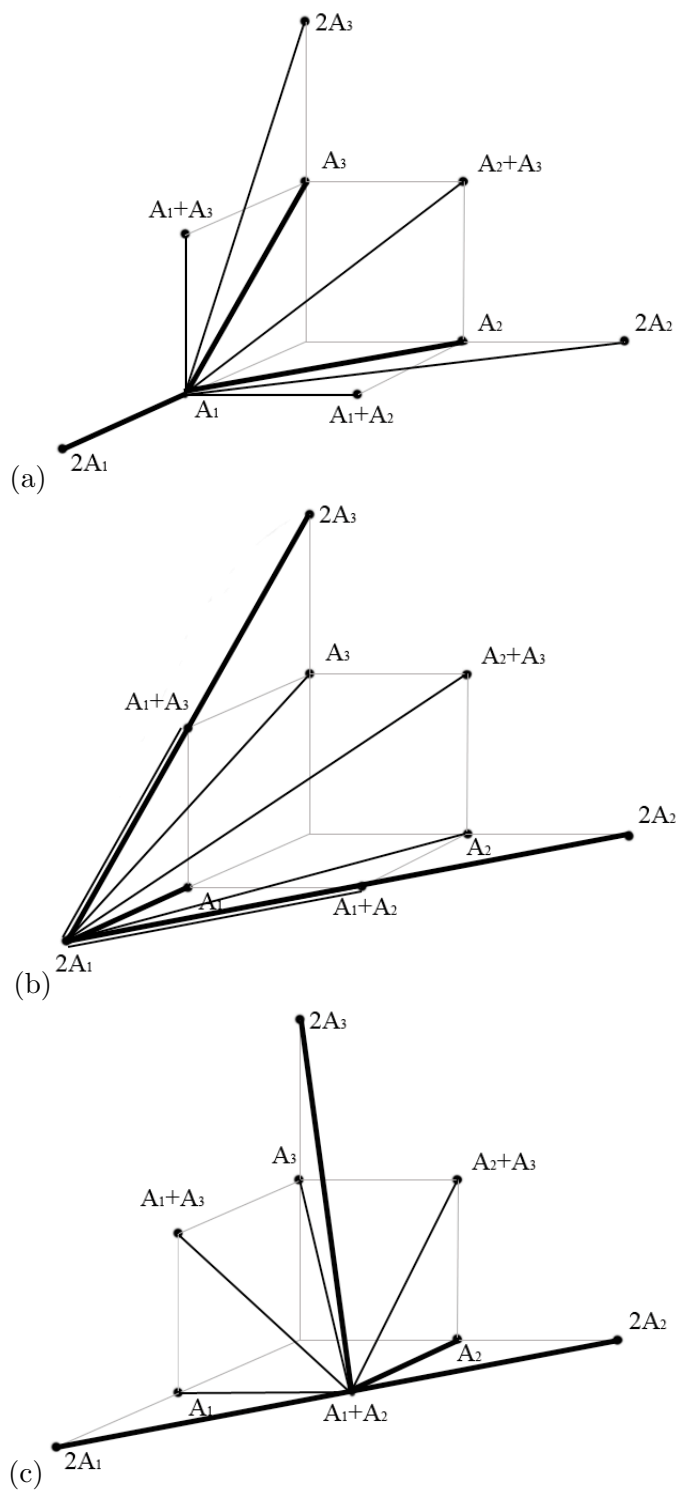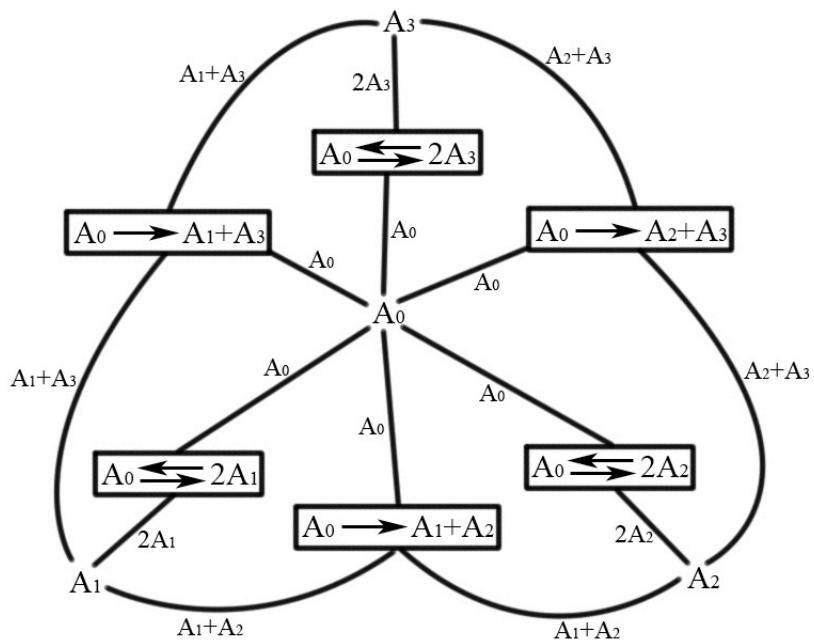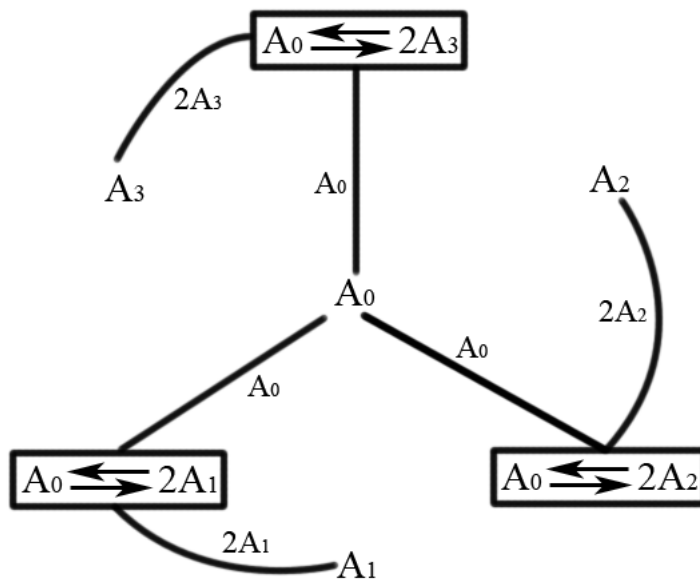FIGURE 1: *Cones corresponding to the reaction networks in (10).*

FIGURE 2: $Cone_{\mathscr{R}}(y)$ for the three possible types of source complexes in (14): (a) reactions with source A_1; (b) reactions with source $2A_1$; (c) reactions with source $A_1 + A_2$.

FIGURE 3: *The SR graph of $\mathscr{R}'$.*

FIGURE 4: *The SR graph of $\mathscr{R}''$.*