Metabolic control analysis in a nutshell

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ABSTRACT

Metabolic control analysis is a powerful quantitative framework for understanding the relationship between the steady-state properties of a (bio)chemical reaction network as a whole and the properties of its component reactions. Although in essence it is a typical sensitivity analysis of a dynamical system, the stoichiometric structure of reaction networks gives it a character of its own. It has proved very useful for both theoretical and experimental analysis of cellular systems, leading to deep insights into matters of control and regulation. This paper attempts to capture in a nutshell the detailed derivation from first principles of all of the important theorems of control analysis starting with the general kinetic model of a reaction network.

1. INTRODUCTION

Like the evolution of life, the development of metabolic control analysis can be likened to a process of tinkering. What now stands as the theoretical body of control analysis is the result of a piecemeal addition to and refinement of theorems presented in the original papers of Kacser and Burns [21, 22] and Heinrich and Rapoport [11]. A landmark paper on the formalisation of control analysis is by Reder [30], although others have provided some formal description from first principles [2, 3, 8, 9, 10, 41]. At present the most complete formalisation can be found in [13]. One may therefore rightly question the need for another treatment. However, reinventing the metaphorical wheel often yields new insights, and it is in this spirit that this paper is offered as a journey of discovery through the algebraical landscape of metabolic control analysis.

We start at the very beginning with the general kinetic model for a network of chemical reactions, and then proceed step by step, doing our best to avoid those unexplained jumps which, although seemingly obvious to experienced mathematicians, leaves us lesser mortals feeling woefully inadequate. Nevertheless, the reader is at least assumed to be acquainted with introductory differential calculus. The great strength and elegance of symbolic matrix algebra is utilised throughout, but there is nothing mysterious about it. The rules of matrix algebra are similar to those of ordinary algebra [37], but be mindful of two things: (i) two matrices can only be multiplied if the number of columns of the first matrix equals the number of rows of the second (an $m \times n$ matrix can only be multiplied by a $n \times k$ matrix; the product will have dimensions $m \times k$), and (ii) matrix multiplication is not commutative, i.e., the product **AB** is usually not equal to **BA**. For those that feel more comfortable with explicit matrix equations, the example provided in Appendix B serves as a starting point.

It must, however, be made clear from the outset that this is neither a literature review, nor a discussion of the theoretical and experimental applications of control analysis or the physical interpretation of control properties. For this the reader is referred to the original literature, perhaps with the excellent monographs [5] and [13] as points of departure. Here only selected key references are supplied.

2. THE KINETIC MODEL

The kinetic model for any (metabolic) network of coupled chemical reactions and transport processes can be written as a set of nonlinear differential equations (see e.g., [30]):

$$\frac{d\mathbf{s}}{dt} = \mathbf{N}\mathbf{v}[\mathbf{s}, \mathbf{p}] \tag{1}$$

where, for a system of n coupled reactions that inter-convert m substances (from here on called 'metabolites'), $\mathbf{s}$ is an m-dimensional column vector of metabolite concentrations, $\mathbf{N}$ is an $m \times n$-dimensional matrix of stoichiometric coefficients (the stoichiometric matrix), $\mathbf{v}$ is an n-dimensional column vector of reaction rates, and $\mathbf{p}$ is a p-dimensional column vector of parameters. Only variable metabolite concentrations are included in $\mathbf{s}$; metabolite concentrations which are buffered externally and can therefore be regarded as constant are considered to be included in the parameter vector $\mathbf{p}$.

In any systemic state the reaction rates $\mathbf{v}$ are functions of both metabolite concentrations $\mathbf{s}$ and parameters $\mathbf{p}$ such as kinetic constants and fixed external concentrations. This is expressed in eqn. 1 by the functional relationship $\mathbf{v} = \mathbf{v}[\mathbf{s}, \mathbf{p}]$.

The structure or topology of the reaction network is embodied in the stoichiometric matrix $\mathbf{N}$. An element c_{ij} of $\mathbf{N}$ is the stoichiometry, usually an integer, with which metabolite S_i participates in reaction j (if S_i is a reactant, $c_{ij} < 0$; if a product, $c_{ij} > 0$; otherwise, $c_{ij} = 0$). Two invariant properties can be extracted from $\mathbf{N}$, namely (i) the conservation relationships that arise when the differential equations are not all linearly independent, and (ii) the steady-state flux relationships [30]. Here we discuss the first; the second will follow once the steady state has been treated.

By Gaussian elimination to row echelon form (see, e.g., [37]) we can determine whether the rows of $\mathbf{N}$ (and, therefore, the differential equations themselves) are linearly independent (see Appendix B for an example). If they are independent then $r = m$, where r is the rank of $\mathbf{N}$ (the number of independent equations). If $r < m$ then there are $m - r$ dependencies among the differential equations. Eliminating $m - r$ dependent rows of $\mathbf{N}$ leaves a reduced stoichiometric matrix, $\mathbf{N_R}$, with r independent rows. $\mathbf{N}$ and $\mathbf{N_R}$ can be related by constructing a *link matrix* $\mathbf{L}$ with dimensions $m \times r$ so that $\mathbf{N} = \mathbf{L}\mathbf{N_R}$ [30]. If $\mathbf{N}$ is re-arranged so that the independent rows

come first, then $\mathbf{L}$ and the concentration vector $\mathbf{s}$ that corresponds to the rows of the re-arranged $\mathbf{N}$ have the structure

$$\mathbf{L} = \begin{bmatrix} \mathbf{I}_r \\ \mathbf{L_0} \end{bmatrix} \qquad \text{and} \qquad \mathbf{s} = \begin{bmatrix} \mathbf{s_i} \\ \mathbf{s_d} \end{bmatrix} \tag{2}$$

where $\mathbf{I}_r$ is an r-dimensional identity matrix and $\mathbf{L_0}$ an $(m-r)\times r$-dimensional matrix that expresses the dependent time derivatives in terms of the independent time derivatives (see eqn. 6 below); $\mathbf{s_i}$ refers to independent and $\mathbf{s_d}$ to dependent concentrations.

Using these relationships the kinetic model in eqn. 1 can be written as

$$\frac{d\mathbf{s}}{dt} = \mathbf{L}\mathbf{N_R}\mathbf{v}[\mathbf{s_i}, \mathbf{s_d}, \mathbf{p}] \tag{3}$$

where the functional relationship $\mathbf{v} = \mathbf{v}[\mathbf{s_i}, \mathbf{s_d}, \mathbf{p}]$ emphasises the fact that the dependencies among the differential equations allows the partitioning of $\mathbf{s}$ into r independent concentrations $\mathbf{s_i}$ and $m-r$ dependent concentrations $\mathbf{s_d}$. Now the kinetic model can be expanded into

$$\frac{d}{dt}\begin{bmatrix} \mathbf{s_i} \\ \mathbf{s_d} \end{bmatrix} = \mathbf{L}\mathbf{N_R}\mathbf{v}[\mathbf{s_i}, \mathbf{s_d}, \mathbf{p}] = \begin{bmatrix} \mathbf{I}_r \\ \mathbf{L_0} \end{bmatrix}\mathbf{N_R}\mathbf{v}[\mathbf{s_i}, \mathbf{s_d}, \mathbf{p}] \tag{4}$$

which can be split into two equations:

$$\frac{d\mathbf{s_i}}{dt} = \mathbf{N_R}\mathbf{v}[\mathbf{s_i}, \mathbf{s_d}, \mathbf{p}] \tag{5}$$

$$\frac{d\mathbf{s_d}}{dt} = \mathbf{L_0}\mathbf{N_R}\mathbf{v}[\mathbf{s_i}, \mathbf{s_d}, \mathbf{p}] = \mathbf{L_0}\frac{d\mathbf{s_i}}{dt} \tag{6}$$

which, when combined, give

$$\frac{d\mathbf{s}}{dt} = \mathbf{L}\frac{d\mathbf{s_i}}{dt} \tag{7}$$

It is clear that if $\mathbf{L_0}$ is known we need only consider the kinetics as expressed by eqn. 5, as eqn. 6 allows the expression of the linear dependencies between the rates of change of metabolite concentrations:

$$\frac{d}{dt}(\mathbf{s_d} - \mathbf{L_0}\mathbf{s_i}) = \mathbf{0} \tag{8}$$

where $\mathbf{0}$ is a null vector (a vector of zeros). This implies that

$$\mathbf{s_d} = \mathbf{L_0}\mathbf{s_i} + \mathbf{T} \tag{9}$$

where $\mathbf{T}$ is an $(m-r)$-dimensional vector of constant (conserved) sums of concentrations. The full concentration vector $\mathbf{s}$ can therefore be expressed as a function of $\mathbf{s_i}$ and $\mathbf{T}$:

$$\mathbf{s} = \begin{bmatrix} \mathbf{s_i} \\ \mathbf{s_d} \end{bmatrix} = \begin{bmatrix} \mathbf{I}_r \\ \mathbf{L_0} \end{bmatrix}\mathbf{s_i} + \begin{bmatrix} \mathbf{0} \\ \mathbf{T} \end{bmatrix} = \mathbf{L}\mathbf{s_i} + \begin{bmatrix} \mathbf{0} \\ \mathbf{T} \end{bmatrix} \tag{10}$$

where $\mathbf{0}$ represents an r-dimensional subvector of zeros.

2.1 Functional relationships in the steady state

In the steady state the kinetic model $d\mathbf{s}/dt = \mathbf{0}$, and the equations simplify to a system of non-linear equations of the form

$$\mathbf{N_R}\mathbf{v}[\mathbf{s_i}, \mathbf{s_d}, \mathbf{p}] = \mathbf{0} \tag{11}$$

When there are no conservation relationships (when $r = m$), the equation system reduces to a slightly simpler form

$$\mathbf{N}\mathbf{v}[\mathbf{s}, \mathbf{p}] = \mathbf{0} \tag{12}$$

but we shall only consider eqn. 11 as it is more general. The solution to eqn. 11 is a vector of independent concentrations

$$\mathbf{s_i} = \mathbf{s_i}[\mathbf{T}, \mathbf{p}] \tag{13}$$

Note that the concentrations are now *steady-state* concentrations (as the context is clear we deem it wise not to confuse things by altering the symbol; from here on $\mathbf{s}$, $\mathbf{s_i}$, and $\mathbf{s_d}$ only refer to steady-state concentrations). Furthermore, the solution can be expanded to a vector of dependent steady-state concentrations, which depends through eqn. 9 on $\mathbf{s_i}$ and $\mathbf{T}$,

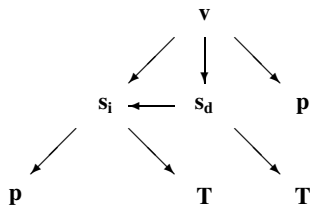
$$\mathbf{s_d} = \mathbf{s_d}[\mathbf{s_i}, \mathbf{T}] \tag{14}$$

and a steady-state reaction rate vector

$$\mathbf{J} = \mathbf{v}[\mathbf{s_i}, \mathbf{s_d}, \mathbf{p}] \tag{15}$$

for which we reserve the special name *flux vector.*

Usually, we cannot solve for the steady-state concentrations and fluxes analytically, although the powerful symbolic manipulators available today (e.g., Mathematica, Maple, Reduce) enlarge the scope of what is possible. Except for the simplest cases, analytical solutions are in any case extremely difficult to interpret. *The central question asked by metabolic control analysis is how the steady-state variables change when the steady-state changes in response to a perturbation in one or more parameters.* In order to answer this question it is necessary to differentiate the steady-state equations with respect to the parameters, and for this we must have an accurate picture of the functional relationships in these equations. A diagrammatic representation makes these nested functional relationships more transparent:



It is clear that the steady-state concentrations $\mathbf{s_i}$ and $\mathbf{s_d}$ as well as the steady-state fluxes $\mathbf{J}$, ultimately depend only on the parameters $\mathbf{p}$ and the conservation sums $\mathbf{T}$. Nevertheless, the intermediary levels of functional dependencies are important when the steady-state equations are differentiated with respect to $\mathbf{p}$ and $\mathbf{T}$. However, before we turn to this topic we complete our structural analysis of $\mathbf{N}$ by considering the relationships that exist between fluxes in the steady state.

2.2 Flux-relationships in the steady state

We showed above that linear dependencies among the rows of $\mathbf{N}$ can be captured in the link matrix $\mathbf{L}$. Similarly, in the steady state when $\mathbf{N_R}\mathbf{v} = \mathbf{0}$ (or $\mathbf{N}\mathbf{v} = \mathbf{0}$, if there are no conservation relationships) their exist dependencies among the columns of $\mathbf{N}$ (or $\mathbf{N_R}$) that can be expressed as

$$\mathbf{N}\mathbf{K} = \mathbf{0} \qquad \text{or} \qquad \mathbf{N_R}\mathbf{K} = \mathbf{0} \tag{16}$$

where $\mathbf{K}$ is the kernel (or nullspace) of $\mathbf{N}$ [30]. Each column of $\mathbf{K}$ is a particular solution to eqn. 16, and the set of columns are linearly independent and therefore span the nullspace. Because each column represents an independent flux, it follows that:

$$\mathbf{J} = \mathbf{K}\mathbf{J_i} \tag{17}$$

where $\mathbf{J}$ is an n-dimensional column vector of all the steady-state fluxes, and $\mathbf{J_i}$ is an $(n-r)$-dimensional column vector of independent fluxes (recall that r is the rank of the stoichiometric matrix). $\mathbf{K}$

therefore has dimensions $n \times (n-r)$. If $\mathbf{K}$ is re-arranged so that the $n-r$ rows that correspond to independent fluxes come first, then the flux vector $\mathbf{J}$ is partitioned into $n-r$ independent fluxes $\mathbf{J_i}$ and r dependent fluxes $\mathbf{J_d}$, and eqn. 17 becomes

$$\begin{bmatrix} \mathbf{J_i} \\ \mathbf{J_d} \end{bmatrix} = \begin{bmatrix} \mathbf{I}_{n-r} \\ \mathbf{K_0} \end{bmatrix} \mathbf{J_i} \tag{18}$$

where $\mathbf{I}$ is an $(n-r)$-dimensional identity matrix and $\mathbf{K_0}$ an $r\times(n-r)$-dimensional matrix that expresses the dependent fluxes in terms of the independent fluxes, $\mathbf{J_d} = \mathbf{K_0}\mathbf{J_i}$.

3. DIFFERENTIATION OF THE STEADY-STATE EQUATION

Because the parameters $\mathbf{p}$ and $\mathbf{T}$ determine the steady-state, any change in these parameters can potentially change the steady state. If the parameter perturbation is small enough, the change from steady state $(\mathbf{s}_1, \mathbf{J}_1)$ to steady state $(\mathbf{s}_2, \mathbf{J}_2)$ is approximated by

$$\mathbf{s}_2 = \mathbf{s}_1 + \frac{\partial \mathbf{s}}{\partial \mathbf{p}}(\mathbf{p}_2 - \mathbf{p}_1) \tag{19}$$

and

$$\mathbf{J}_2 = \mathbf{J}_1 + \frac{\partial \mathbf{J}}{\partial \mathbf{p}}(\mathbf{p}_2 - \mathbf{p}_1) \tag{20}$$

the first two terms in the so-called multivariate Taylor expansion. Similar equations for perturbations in $\mathbf{T}$ can be obtained by replacing $\mathbf{p}$ by $\mathbf{T}$. The matrices of partial derivatives $\partial\mathbf{s}/\partial\mathbf{p}$, $\partial\mathbf{J}/\partial\mathbf{p}$, $\partial\mathbf{s}/\partial\mathbf{T}$, and $\partial\mathbf{J}/\partial\mathbf{T}$ are thus of great interest and will be obtained next.

Readers will be familiar with the differentiation of an explicit mathematical equation of the form $y = f(x)$ with respect to x, or if, as is the case here, y is a function of more than one variable $y = f(x, z)$, partial differentiation with respect to either x or z or both at the same time. However, we are working not with simple scalar variables, but with vector variables. In addition, equations such as eqn. 11 are implicit functions. Fortunately, the extension to vector variables and implicit functions is not difficult at all. Let us first get a feel for this process by differentiating eqn. 14, the explicit function $\mathbf{s_d} = \mathbf{L_0}\mathbf{s_i}[\mathbf{T}, \mathbf{p}] + \mathbf{T}$. We use the part of the diagram of functional relationships given above that starts at $\mathbf{s_d}$ as a guide. First, let us partially differentiate $\mathbf{s_d}$ with respect to $\mathbf{s_i}$ (at constant $\mathbf{T}$ and $\mathbf{p}$):

$$\left(\frac{\partial \mathbf{s_d}}{\partial \mathbf{s_i}}\right)_{\mathbf{T},\mathbf{p}} = \mathbf{L_0} \tag{21}$$

When we differentiate with respect to $\mathbf{T}$ at constant $\mathbf{p}$ there are two routes from $\mathbf{s_d}$ to $\mathbf{T}$, one via $\mathbf{s_i}$ and one direct:

$$\left(\frac{\partial \mathbf{s_d}}{\partial \mathbf{T}}\right)_{\mathbf{p}} = \left(\frac{\partial \mathbf{s_d}}{\partial \mathbf{s_i}}\right)_{\mathbf{T},\mathbf{p}} \left(\frac{\partial \mathbf{s_i}}{\partial \mathbf{T}}\right)_{\mathbf{p}} + \left(\frac{\partial \mathbf{s_d}}{\partial \mathbf{T}}\right)_{\mathbf{s_i},\mathbf{p}} \tag{22}$$

Inserting eqn. 21 we obtain

$$\left(\frac{\partial \mathbf{s_d}}{\partial \mathbf{T}}\right)_{\mathbf{p}} = \mathbf{L_0}\left(\frac{\partial \mathbf{s_i}}{\partial \mathbf{T}}\right)_{\mathbf{p}} + \mathbf{I}_{m-r} \tag{23}$$

Note how we use the chain rule to follow the different branches of the tree structure of the diagram of functional relationships. Because we are differentiating with respect to vectors, all the dimensions have to be consistent; this is why an identity matrix $\mathbf{I}_{m-r}$ appears rather than a 1. Finally, we differentiate with respect to $\mathbf{p}$ at constant $\mathbf{T}$:

$$\left(\frac{\partial \mathbf{s_d}}{\partial \mathbf{p}}\right)_{\mathbf{T}} = \left(\frac{\partial \mathbf{s_d}}{\partial \mathbf{s_i}}\right)_{\mathbf{T},\mathbf{p}} \left(\frac{\partial \mathbf{s_i}}{\partial \mathbf{p}}\right)_{\mathbf{T}} = \mathbf{L_0}\left(\frac{\partial \mathbf{s_i}}{\partial \mathbf{p}}\right)_{\mathbf{T}} \tag{24}$$

We shall need all three of these equations when we next proceed to differentiate the kinetic model in steady state. We first differentiate with respect to $\mathbf{p}$ at constant $\mathbf{T}$, and then with respect to $\mathbf{T}$ at constant $\mathbf{p}$.

Case 1: $d\mathbf{p} \neq \mathbf{0}$, $d\mathbf{T} = \mathbf{0}$

Eqn. 11 is an implicit equation of the form $f(x, y, z) = 0$. In general, to obtain, say, $\partial y/\partial x$ one could in principle solve for y and partially differentiate with respect to x while keeping z constant. This is often impossible, but there is, fortunately, a much simpler way around this, namely *implicit differentiation*

$$df = \left(\frac{\partial f}{\partial x}\right)_{y,z} dx + \left(\frac{\partial f}{\partial y}\right)_{x,z} dy + \left(\frac{\partial f}{\partial z}\right)_{x,y} dz = 0 \tag{25}$$

where df is called the total differential. If only one variable, say x, is considered to change at constant y and z, then one obtains

$$\left(\frac{\partial f}{\partial x}\right)_{y,z} + \left(\frac{\partial f}{\partial y}\right)_{x,z}\left(\frac{\partial y}{\partial x}\right)_{z} + \left(\frac{\partial f}{\partial z}\right)_{x,y}\left(\frac{\partial z}{\partial x}\right)_{y} = 0 \tag{26}$$

To differentiate Eqn. 11 with respect to $\mathbf{p}$ we use this technique in combination with the chain rule to traverse the three routes from $\mathbf{v}$ to $\mathbf{p}$ on the diagram of functional relationships:

$$\mathbf{N_R}\left[\left(\frac{\partial \mathbf{v}}{\partial \mathbf{s_i}}\right)_{\mathbf{s_d},\mathbf{T},\mathbf{p}}\left(\frac{\partial \mathbf{s_i}}{\partial \mathbf{p}}\right)_{\mathbf{T}} + \left(\frac{\partial \mathbf{v}}{\partial \mathbf{s_d}}\right)_{\mathbf{s_i},\mathbf{T},\mathbf{p}}\left(\frac{\partial \mathbf{s_d}}{\partial \mathbf{s_i}}\right)_{\mathbf{T},\mathbf{p}}\left(\frac{\partial \mathbf{s_i}}{\partial \mathbf{p}}\right)_{\mathbf{T}} + \left(\frac{\partial \mathbf{v}}{\partial \mathbf{p}}\right)_{\mathbf{T}}\right] = \mathbf{0} \tag{27}$$

where $\mathbf{0}$ is a null matrix. Inserting eqn. 21 and collecting the first two terms in the square brackets we obtain

$$\mathbf{N_R}\begin{bmatrix} \frac{\partial \mathbf{v}}{\partial \mathbf{s_i}} & \frac{\partial \mathbf{v}}{\partial \mathbf{s_d}} \end{bmatrix}\begin{bmatrix} \mathbf{I}_r \\ \mathbf{L_0} \end{bmatrix}\left(\frac{\partial \mathbf{s_i}}{\partial \mathbf{p}}\right)_{\mathbf{T}} + \mathbf{N_R}\left(\frac{\partial \mathbf{v}}{\partial \mathbf{p}}\right)_{\mathbf{T}} = \mathbf{0} \tag{28}$$

By definition the column vector of matrices is $\mathbf{L}$. The partitioned matrix $[\frac{\partial \mathbf{v}}{\partial \mathbf{s_i}} \;\; \frac{\partial \mathbf{v}}{\partial \mathbf{s_d}}]$ is the matrix $(\partial\mathbf{v}/\partial\mathbf{s})_{\mathbf{T},\mathbf{p}}$ of partial derivatives of reaction rate functions with respect to the individual concentrations in $\mathbf{s}$. In control analysis these partial derivatives are called *elasticity coefficients*, defined as $\bar{\varepsilon}^{v_k}_{s_j} = \partial v_k/\partial s_j$. The above matrix of elasticity coefficients is symbolised with $\bar{\boldsymbol{\varepsilon}}_{\mathbf{s}}$. Similarly, $(\partial\mathbf{v}/\partial\mathbf{p})_{\mathbf{T}}$ is a matrix of elasticity coefficients with respect to $\mathbf{p}$, symbolised by $\bar{\boldsymbol{\varepsilon}}_{\mathbf{p}}$. The bar in $\bar{\boldsymbol{\varepsilon}}$ reminds us that these are ordinary partial derivatives, not the normalised (scaled) partial derivatives which are usually used in control analysis and which we shall encountered further on (and for which we shall use the unbarred symbol; *this distinction between barred (non-normalised) and unbarred (normalised) symbols is made throughout the paper for all the coefficients of control analysis and their matrices*). Using this symbolism eqn. 28 is written as

$$\mathbf{N_R}\bar{\boldsymbol{\varepsilon}}_{\mathbf{s}}\mathbf{L}\left(\frac{\partial \mathbf{s_i}}{\partial \mathbf{p}}\right)_{\mathbf{T}} + \mathbf{N_R}\bar{\boldsymbol{\varepsilon}}_{\mathbf{p}} = \mathbf{0} \tag{29}$$

The steady-state eqn. 15 for fluxes is explicit. Differentiation with respect to $\mathbf{p}$ yields:

$$\left(\frac{\partial \mathbf{J}}{\partial \mathbf{p}}\right)_{\mathbf{T}} = \left(\frac{\partial \mathbf{v}}{\partial \mathbf{s_i}}\right)_{\mathbf{s_d},\mathbf{T},\mathbf{p}}\left(\frac{\partial \mathbf{s_i}}{\partial \mathbf{p}}\right)_{\mathbf{T}} + \left(\frac{\partial \mathbf{v}}{\partial \mathbf{s_d}}\right)_{\mathbf{s_i},\mathbf{T},\mathbf{p}}\left(\frac{\partial \mathbf{s_d}}{\partial \mathbf{s_i}}\right)_{\mathbf{T},\mathbf{p}}\left(\frac{\partial \mathbf{s_i}}{\partial \mathbf{p}}\right)_{\mathbf{T}} + \left(\frac{\partial \mathbf{v}}{\partial \mathbf{p}}\right)_{\mathbf{T}} \tag{30}$$

This expression is identical to the sum of terms within the square brackets of eqn. 27. Substituting and collecting terms as before

gives

$$\left(\frac{\partial \mathbf{J}}{\partial \mathbf{p}}\right)_{\mathbf{T}} = \bar{\boldsymbol{\varepsilon}}_{\mathbf{s}}\mathbf{L}\left(\frac{\partial \mathbf{s_i}}{\partial \mathbf{p}}\right)_{\mathbf{T}} + \bar{\boldsymbol{\varepsilon}}_{\mathbf{p}} \tag{31}$$

Case 2: $d\mathbf{p} = \mathbf{0}$, $d\mathbf{T} \neq \mathbf{0}$

Now we implicitly differentiate eqn. 11 with respect to **T** at constant **p**. On the diagram of functional relationships there are two routes from **v** to **T**; one of them branches at $\mathbf{s_d}$ into two subroutes to **T**. We differentiate in two steps to avoid getting things mixed up—first we differentiate to the level of $\mathbf{s_i}$ and $\mathbf{s_d}$:

$$\mathbf{N}_{\mathbf{R}}\left[\left(\frac{\partial \mathbf{v}}{\partial \mathbf{s_i}}\right)_{\mathbf{s_d},\mathbf{T},\mathbf{p}}\left(\frac{\partial \mathbf{s_i}}{\partial \mathbf{T}}\right)_{\mathbf{p}} + \left(\frac{\partial \mathbf{v}}{\partial \mathbf{s_d}}\right)_{\mathbf{s_i},\mathbf{T},\mathbf{p}}\left(\frac{\partial \mathbf{s_d}}{\partial \mathbf{T}}\right)_{\mathbf{p}}\right] = \mathbf{0} \tag{32}$$

Now we must take care of the two routes from $\mathbf{s_d}$ to **T**. In fact, we have already done this in eqn. 22 so we just substitute:

$$\mathbf{N}_{\mathbf{R}}\left[\left(\frac{\partial \mathbf{v}}{\partial \mathbf{s_i}}\right)_{\mathbf{s_d},\mathbf{T},\mathbf{p}}\left(\frac{\partial \mathbf{s_i}}{\partial \mathbf{T}}\right)_{\mathbf{p}} + \left(\frac{\partial \mathbf{v}}{\partial \mathbf{s_d}}\right)_{\mathbf{s_i},\mathbf{T},\mathbf{p}}\left[\mathbf{L}_0\left(\frac{\partial \mathbf{s_i}}{\partial \mathbf{T}}\right)_{\mathbf{p}} + \mathbf{I}_{m-r}\right]\right] = \mathbf{0} \tag{33}$$

Multiplying out and collecting the first two terms as before gives

$$\mathbf{N}_{\mathbf{R}}\left[\bar{\boldsymbol{\varepsilon}}_{\mathbf{s}}\mathbf{L}\left(\frac{\partial \mathbf{s_i}}{\partial \mathbf{T}}\right)_{\mathbf{p}} + \bar{\boldsymbol{\varepsilon}}_{\mathbf{s_d}}\right] = \mathbf{0} \tag{34}$$

Explicit differentiation of eqn. 15 with respect to **T** at constant **p** gives:

$$\left(\frac{\partial \mathbf{J}}{\partial \mathbf{T}}\right)_{\mathbf{p}} = \left(\frac{\partial \mathbf{v}}{\partial \mathbf{s_i}}\right)_{\mathbf{s_d},\mathbf{T},\mathbf{p}}\left(\frac{\partial \mathbf{s_i}}{\partial \mathbf{T}}\right)_{\mathbf{p}} + \left(\frac{\partial \mathbf{v}}{\partial \mathbf{s_d}}\right)_{\mathbf{s_i},\mathbf{T},\mathbf{p}}\left(\frac{\partial \mathbf{s_d}}{\partial \mathbf{T}}\right)_{\mathbf{p}} \tag{35}$$

We proceed exactly as in eqns. 33 and 34 to obtain

$$\left(\frac{\partial \mathbf{J}}{\partial \mathbf{T}}\right)_{\mathbf{p}} = \bar{\boldsymbol{\varepsilon}}_{\mathbf{s}}\mathbf{L}\left(\frac{\partial \mathbf{s_i}}{\partial \mathbf{T}}\right)_{\mathbf{p}} + \bar{\boldsymbol{\varepsilon}}_{\mathbf{s_d}} \tag{36}$$

This concludes the differentiation of the steady-state equations.

4. METABOLIC CONTROL ANALYSIS

We are now in a position to derive the basic definitions of and relationships between all the different matrices of the coefficients of metabolic control analysis (the boxed equations in the rest of this section).

4.1 Concentration response with respect to p

The matrix product $\mathbf{N}_{\mathbf{R}}\bar{\boldsymbol{\varepsilon}}_{\mathbf{s}}\mathbf{L}$, which appears in eqn. 29, is the so-called *Jacobian matrix*, which we symbolise with **M**. The nature and significance of the Jacobian matrix is explained in Appendix A. Here we just note that if a steady state exists the Jacobian matrix is invertible. From here on, for the sake of brevity, we leave out the subscripts that indicate which vectors remain constant during differentiation. In this spirit, eqn. 29 is now written as

$$\frac{\partial \mathbf{s_i}}{\partial \mathbf{p}} = -\mathbf{M}^{-1}\mathbf{N}_{\mathbf{R}}\bar{\boldsymbol{\varepsilon}}_{\mathbf{p}} \tag{37}$$

From eqn. 24 it follows that

$$\frac{\partial \mathbf{s_d}}{\partial \mathbf{p}} = \mathbf{L}_0\frac{\partial \mathbf{s_i}}{\partial \mathbf{p}} = -\mathbf{L}_0\mathbf{M}^{-1}\mathbf{N}_{\mathbf{R}}\bar{\boldsymbol{\varepsilon}}_{\mathbf{p}} \tag{38}$$

Combining the eqns. 37 and 38 gives

$$\begin{bmatrix} \frac{\partial \mathbf{s_i}}{\partial \mathbf{p}} \\ \frac{\partial \mathbf{s_d}}{\partial \mathbf{p}} \end{bmatrix} = -\begin{bmatrix} \mathbf{I}_r \\ \mathbf{L}_0 \end{bmatrix}\mathbf{M}^{-1}\mathbf{N}_{\mathbf{R}}\bar{\boldsymbol{\varepsilon}}_{\mathbf{p}} \tag{39}$$

which simplifies to

$$\frac{\partial \mathbf{s}}{\partial \mathbf{p}} = (-\mathbf{L}\mathbf{M}^{-1}\mathbf{N}_{\mathbf{R}})\bar{\boldsymbol{\varepsilon}}_{\mathbf{p}} \tag{40}$$

We have therefore obtained the first of the matrices of partial derivatives that we seek. The elements of this matrix are *concentration-response coefficients*, defined as $R^{s_j}_{p_k} = \partial s_j/\partial p_k$; they quantify the steady-state response in a metabolite concentration s_j to a perturbation in parameter p_k. The matrix $\partial\mathbf{s}/\partial\mathbf{p}$ will be symbolised by $\bar{\mathbf{R}}^{\mathbf{s}}_{\mathbf{p}}$.

4.2 Concentration-control coefficients

What are the elements of the matrix $-\mathbf{L}\mathbf{M}^{-1}\mathbf{N}_{\mathbf{R}}$, or in more explicit form, $-\mathbf{L}(\mathbf{N}_{\mathbf{R}}\bar{\boldsymbol{\varepsilon}}_{\mathbf{s}}\mathbf{L})^{-1}\mathbf{N}_{\mathbf{R}}$? Besides **L** and $\mathbf{N}_{\mathbf{R}}$, which are integer matrices, this matrix contains only partial derivatives of the rates with respect to the steady-state concentrations, i.e., elasticity coefficients; nothing in the matrix depends explicitly on **p** or **T**. Now, consider a set of parameters such that each uniquely affects a single reaction, i.e., in the elasticity matrix $\bar{\boldsymbol{\varepsilon}}_{\mathbf{p}}$

$$\frac{\partial v_k}{\partial p_k} \neq 0 \quad \text{and} \quad \frac{\partial v_k}{\partial p_l} = 0 \quad \text{for} \quad k \neq l \tag{41}$$

This means that $\bar{\boldsymbol{\varepsilon}}_{\mathbf{p}}$ is now a diagonal matrix (only the diagonal elements $\partial v_k/\partial p_k$ are non-zero). The inverse of a diagonal matrix is again a diagonal matrix but with all diagonal elements in reciprocal form. Therefore, by multiplying both sides of eqn. 40 with this inverse leads to a matrix expression for $-\mathbf{L}\mathbf{M}^{-1}\mathbf{N}_{\mathbf{R}}$ in which an element in row j and column k is

$$\frac{\partial s_j}{\partial p_k}\Big/\frac{\partial v_k}{\partial p_k} \tag{42}$$

This is, in fact, the fundamental definition of a *concentration-control coefficient*:

$$\bar{C}^{s_j}_{k} = \frac{\partial s_j}{\partial p_k}\Big/\frac{\partial v_k}{\partial p_k} \tag{43}$$

The matrix $-\mathbf{L}\mathbf{M}^{-1}\mathbf{N}_{\mathbf{R}}$ is therefore the matrix of concentration-control coefficients

$$\boxed{\bar{\mathbf{C}}^{\mathbf{s}} = -\mathbf{L}\mathbf{M}^{-1}\mathbf{N}_{\mathbf{R}}} \tag{44}$$

The matrix $\partial\mathbf{s}/\partial\mathbf{p}$ can be recognised as a matrix of concentration-response coefficients $\bar{\mathbf{R}}^{\mathbf{s}}_{\mathbf{p}}$. Eqn. 40 is therefore a statement of the *partitioned concentration-response property* of metabolic systems:

$$\boxed{\bar{\mathbf{R}}^{\mathbf{s}}_{\mathbf{p}} = \bar{\mathbf{C}}^{\mathbf{s}}\bar{\boldsymbol{\varepsilon}}_{\mathbf{p}}} \tag{45}$$

4.3 Flux-response and control coefficients

Differentiation of the steady-state flux equation with respect to **p** led to eqn. 31. Inserting eqn. 37 we obtain

$$\frac{\partial \mathbf{J}}{\partial \mathbf{p}} = \bar{\boldsymbol{\varepsilon}}_{\mathbf{s}}\left(-\mathbf{L}\mathbf{M}^{-1}\mathbf{N}_{\mathbf{R}}\right)\bar{\boldsymbol{\varepsilon}}_{\mathbf{p}} + \bar{\boldsymbol{\varepsilon}}_{\mathbf{p}} \tag{46}$$

From eqn. 44 we recognise the bracketed term as $\bar{\mathbf{C}}^{\mathbf{s}}$, so that

$$\frac{\partial \mathbf{J}}{\partial \mathbf{p}} = \left(\bar{\boldsymbol{\varepsilon}}_{\mathbf{s}}\bar{\mathbf{C}}^{\mathbf{s}} + \mathbf{I}_n\right)\bar{\boldsymbol{\varepsilon}}_{\mathbf{p}} \tag{47}$$

Similar to the previous section, an element in the ith row and kth column of $\bar{\boldsymbol{\varepsilon}}_{\mathbf{s}}\bar{\mathbf{C}}^{\mathbf{s}} + \mathbf{I}_n$ can be seen to be a *flux-control coefficient* of reaction k:

$$\bar{C}^{J_i}_{k} = \frac{\partial J_i}{\partial p_k}\Big/\frac{\partial v_k}{\partial p_k} \tag{48}$$

so that the matrix of flux-control coefficients is defined as

$$\boxed{\bar{\mathbf{C}}^{\mathbf{J}} = \bar{\boldsymbol{\varepsilon}}_{\mathbf{s}}\bar{\mathbf{C}}^{\mathbf{s}} + \mathbf{I}_n} \tag{49}$$

The matrix $\partial\mathbf{J}/\partial\mathbf{p}$ can be recognised as a matrix $\bar{\mathbf{R}}^{\mathbf{J}}_{\mathbf{p}}$ of *flux-response coefficients*, individually defined as $R^{J_i}_{p_k} = \partial J_i/\partial p_k$, that quantify the steady-state response in a flux J_i to a perturbation in parameter p_k. Eqn. 47 is therefore a statement of the *partitioned flux-response property* of metabolic systems:

$$\boxed{\bar{\mathbf{R}}^{\mathbf{J}}_{\mathbf{p}} = \bar{\mathbf{C}}^{\mathbf{J}}\bar{\boldsymbol{\varepsilon}}_{\mathbf{p}}} \tag{50}$$

4.4 Concentration-response with respect to T

Differentiation of eqn. 11 with respect to $\mathbf{T}$ led to eqn. 34, which, if $\partial\mathbf{s_i}/\partial\mathbf{T}$ containing concentration-response coefficients with respect to the conservation sums is symbolised by $\bar{\mathbf{R}}^{\mathbf{s_i}}_{\mathbf{T}}$, can be written as

$$\mathbf{N_R}\bar{\boldsymbol{\varepsilon}}_{\mathbf{s}}\mathbf{L}\bar{\mathbf{R}}^{\mathbf{s_i}}_{\mathbf{T}} + \mathbf{N_R}\bar{\boldsymbol{\varepsilon}}_{\mathbf{s_d}} = \mathbf{0} \tag{51}$$

which, using $\mathbf{N_R}\bar{\boldsymbol{\varepsilon}}_{\mathbf{s}}\mathbf{L} = \mathbf{M}$ and re-arranging gives

$$\bar{\mathbf{R}}^{\mathbf{s_i}}_{\mathbf{T}} = -\mathbf{M}^{-1}\mathbf{N_R}\bar{\boldsymbol{\varepsilon}}_{\mathbf{s_d}} \tag{52}$$

Eqn. 23 can be written as

$$\bar{\mathbf{R}}^{\mathbf{s_d}}_{\mathbf{T}} = \mathbf{L_0}\bar{\mathbf{R}}^{\mathbf{s_i}}_{\mathbf{T}} + \mathbf{I}_{m-r} \tag{53}$$

which, inserting eqn. 52, is

$$\bar{\mathbf{R}}^{\mathbf{s_d}}_{\mathbf{T}} = -\mathbf{L_0}\mathbf{M}^{-1}\mathbf{N_R}\bar{\boldsymbol{\varepsilon}}_{\mathbf{s_d}} + \mathbf{I}_{m-r} \tag{54}$$

Combining eqns. 52 and 54 gives

$$\begin{bmatrix} \bar{\mathbf{R}}^{\mathbf{s_i}}_{\mathbf{T}} \\ \bar{\mathbf{R}}^{\mathbf{s_d}}_{\mathbf{T}} \end{bmatrix} = -\begin{bmatrix} \mathbf{I}_r \\ \mathbf{L_0} \end{bmatrix}\mathbf{M}^{-1}\mathbf{N_R}\bar{\boldsymbol{\varepsilon}}_{\mathbf{s_d}} + \begin{bmatrix} \mathbf{0} \\ \mathbf{I}_{m-r} \end{bmatrix} \tag{55}$$

which reduces to

$$\bar{\mathbf{R}}^{\mathbf{s}}_{\mathbf{T}} = -\mathbf{L}\mathbf{M}^{-1}\mathbf{N_R}\bar{\boldsymbol{\varepsilon}}_{\mathbf{s_d}} + \begin{bmatrix} \mathbf{0} \\ \mathbf{I}_{m-r} \end{bmatrix} \tag{56}$$

As $-\mathbf{L}\mathbf{M}^{-1}\mathbf{N_R} = \bar{\mathbf{C}}^{\mathbf{s}}$ we get

$$\bar{\mathbf{R}}^{\mathbf{s}}_{\mathbf{T}} = \bar{\mathbf{C}}^{\mathbf{s}}\bar{\boldsymbol{\varepsilon}}_{\mathbf{s_d}} + \begin{bmatrix} \mathbf{0} \\ \mathbf{I}_{m-r} \end{bmatrix} \tag{57}$$

Finally, we prefer to write this equation in terms of the full elasticity matrix. This can be done if we realise that

$$\bar{\boldsymbol{\varepsilon}}_{\mathbf{s_d}} = \begin{bmatrix} \bar{\boldsymbol{\varepsilon}}_{\mathbf{s_i}} & \bar{\boldsymbol{\varepsilon}}_{\mathbf{s_d}} \end{bmatrix}\begin{bmatrix} \mathbf{0} \\ \mathbf{I}_{m-r} \end{bmatrix} = \bar{\boldsymbol{\varepsilon}}_{\mathbf{s}}\begin{bmatrix} \mathbf{0} \\ \mathbf{I}_{m-r} \end{bmatrix} \tag{58}$$

Therefore

$$\boxed{\bar{\mathbf{R}}^{\mathbf{s}}_{\mathbf{T}} = (\bar{\mathbf{C}}^{\mathbf{s}}\bar{\boldsymbol{\varepsilon}}_{\mathbf{s}} + \mathbf{I}_m)\begin{bmatrix} \mathbf{0} \\ \mathbf{I}_{m-r} \end{bmatrix}} \tag{59}$$

The righthand matrix singles out the dependent metabolites that are each unique to a different conservation equation, ensuring that each conservation sum is perturbed independently.

4.5 Flux-response with respect to T

Differentiation of eqn. 15 with respect to $\mathbf{T}$ led to eqn. 36, which, if $\partial\mathbf{J}/\partial\mathbf{T}$ containing flux-response coefficients with respect to the conservation sums is symbolised by $\bar{\mathbf{R}}^{\mathbf{J}}_{\mathbf{T}}$, can be re-written as

$$\bar{\mathbf{R}}^{\mathbf{J}}_{\mathbf{T}} = \bar{\boldsymbol{\varepsilon}}_{\mathbf{s}}\mathbf{L}\bar{\mathbf{R}}^{\mathbf{s_i}}_{\mathbf{T}} + \bar{\boldsymbol{\varepsilon}}_{\mathbf{s_d}} \tag{60}$$

We can now insert the expression for $\bar{\mathbf{R}}^{\mathbf{s_i}}_{\mathbf{T}}$ in eqn. 52 to give

$$\bar{\mathbf{R}}^{\mathbf{J}}_{\mathbf{T}} = \bar{\boldsymbol{\varepsilon}}_{\mathbf{s}}(-\mathbf{L}\mathbf{M}^{-1}\mathbf{N_R})\bar{\boldsymbol{\varepsilon}}_{\mathbf{s_d}} + \bar{\boldsymbol{\varepsilon}}_{\mathbf{s_d}} \tag{61}$$

As $\bar{\mathbf{C}}^{\mathbf{s}} = -\mathbf{L}\mathbf{M}^{-1}\mathbf{N_R}$ we obtain

$$\bar{\mathbf{R}}^{\mathbf{J}}_{\mathbf{T}} = (\bar{\boldsymbol{\varepsilon}}_{\mathbf{s}}\bar{\mathbf{C}}^{\mathbf{s}} + \mathbf{I}_n)\bar{\boldsymbol{\varepsilon}}_{\mathbf{s_d}} \tag{62}$$

The bracketed term is the defining expression for $\bar{\mathbf{C}}^{\mathbf{J}}$ (eqn. 49). Therefore,

$$\bar{\mathbf{R}}^{\mathbf{J}}_{\mathbf{T}} = \bar{\mathbf{C}}^{\mathbf{J}}\bar{\boldsymbol{\varepsilon}}_{\mathbf{s_d}} \tag{63}$$

As before, we rather write this in terms of the full elasticity matrix

$$\boxed{\bar{\mathbf{R}}^{\mathbf{J}}_{\mathbf{T}} = \bar{\mathbf{C}}^{\mathbf{J}}\bar{\boldsymbol{\varepsilon}}_{\mathbf{s}}\begin{bmatrix} \mathbf{0} \\ \mathbf{I}_{m-r} \end{bmatrix}} \tag{64}$$

It is possible to express $\bar{\mathbf{R}}^{\mathbf{J}}_{\mathbf{T}}$ in terms of $\bar{\mathbf{R}}^{\mathbf{s}}_{\mathbf{T}}$. Inserting eqn. 49 into eqn. 64 gives

$$\bar{\mathbf{R}}^{\mathbf{J}}_{\mathbf{T}} = (\bar{\boldsymbol{\varepsilon}}_{\mathbf{s}}\bar{\mathbf{C}}^{\mathbf{s}} + \mathbf{I}_n)\bar{\boldsymbol{\varepsilon}}_{\mathbf{s}}\begin{bmatrix} \mathbf{0} \\ \mathbf{I}_{m-r} \end{bmatrix} \tag{65}$$

Multiplying the first RHS product out and recollecting terms gives

$$\bar{\mathbf{R}}^{\mathbf{J}}_{\mathbf{T}} = \bar{\boldsymbol{\varepsilon}}_{\mathbf{s}}(\bar{\mathbf{C}}^{\mathbf{s}}\bar{\boldsymbol{\varepsilon}}_{\mathbf{s}} + \mathbf{I}_m)\begin{bmatrix} \mathbf{0} \\ \mathbf{I}_{m-r} \end{bmatrix} \tag{66}$$

Using eqn. 59 we finally get [1]

$$\boxed{\bar{\mathbf{R}}^{\mathbf{J}}_{\mathbf{T}} = \bar{\boldsymbol{\varepsilon}}_{\mathbf{s}}\bar{\mathbf{R}}^{\mathbf{s}}_{\mathbf{T}}} \tag{67}$$

4.6 Normalising the central equations

In control analysis the use of the dimensionless normalised form of the control and elasticity coefficients is generally preferred [13, 14]. With one trivial exception, the basic equations developed in the previous sections look exactly the same in normalised form, provided that the $\mathbf{K}$, $\mathbf{L}$, $\mathbf{N_R}$ and $\mathbf{M}$-matrices and are scaled appropriately. To do this we define the diagonal matrices $\mathbf{D}^{\mathbf{J}}$ and $\mathbf{D}^{\mathbf{s}}$ which respectively have the steady-state fluxes and concentrations on their diagonal (just as with the coefficient matrices the flux and concentration vectors are arranged so that the independent variables come first, the dependent variables second). Their inverses $(\mathbf{D}^{\mathbf{J}})^{-1}$ and $(\mathbf{D}^{\mathbf{s}})^{-1}$ have inverse fluxes and inverse steady-state concentrations on their diagonals. Similarly, we define $\mathbf{D}^{\mathbf{s_i}}$, $\mathbf{D}^{\mathbf{p}}$, and $\mathbf{D}^{\mathbf{T}}$. Using these diagonal matrices, the matrices that occur in the control-matrix equation are scaled as follows (note that the absence of a bar denotes normalised matrices):

$$\begin{aligned}
\mathbf{C}^{\mathbf{J}} &= (\mathbf{D}^{\mathbf{J}})^{-1}\cdot\bar{\mathbf{C}}^{\mathbf{J}}\cdot\mathbf{D}^{\mathbf{J}} && (68)\\
\mathbf{C}^{\mathbf{s}} &= (\mathbf{D}^{\mathbf{s}})^{-1}\cdot\bar{\mathbf{C}}^{\mathbf{s}}\cdot\mathbf{D}^{\mathbf{J}} && (69)\\
\boldsymbol{\varepsilon}_{\mathbf{s}} &= (\mathbf{D}^{\mathbf{J}})^{-1}\cdot\bar{\boldsymbol{\varepsilon}}_{\mathbf{s}}\cdot\mathbf{D}^{\mathbf{s}} && (70)\\
\mathbf{R}^{\mathbf{J}}_{\mathbf{p}} &= (\mathbf{D}^{\mathbf{J}})^{-1}\cdot\bar{\mathbf{R}}^{\mathbf{J}}_{\mathbf{p}}\cdot\mathbf{D}^{\mathbf{p}} && (71)\\
\mathbf{R}^{\mathbf{s}}_{\mathbf{p}} &= (\mathbf{D}^{\mathbf{s}})^{-1}\cdot\bar{\mathbf{R}}^{\mathbf{s}}_{\mathbf{p}}\cdot\mathbf{D}^{\mathbf{p}} && (72)\\
\mathbf{R}^{\mathbf{J}}_{\mathbf{T}} &= (\mathbf{D}^{\mathbf{J}})^{-1}\cdot\bar{\mathbf{R}}^{\mathbf{J}}_{\mathbf{T}}\cdot\mathbf{D}^{\mathbf{T}} && (73)\\
\mathbf{R}^{\mathbf{s}}_{\mathbf{T}} &= (\mathbf{D}^{\mathbf{s}})^{-1}\cdot\bar{\mathbf{R}}^{\mathbf{s}}_{\mathbf{T}}\cdot\mathbf{D}^{\mathbf{T}} && (74)\\
\mathcal{L} &= (\mathbf{D}^{\mathbf{s}})^{-1}\cdot\mathbf{L}\cdot\mathbf{D}^{\mathbf{s_i}} && (75)\\
\mathcal{K} &= (\mathbf{D}^{\mathbf{J}})^{-1}\cdot\mathbf{K}\cdot\mathbf{D}^{\mathbf{J_i}} && (76)\\
\mathcal{N}_{\mathbf{R}} &= (\mathbf{D}^{\mathbf{s_i}})^{-1}\cdot\mathbf{N_R}\cdot\mathbf{D}^{\mathbf{J}} && (77)\\
\mathcal{M} &= (\mathbf{D}^{\mathbf{s_i}})^{-1}\cdot\mathbf{M}\cdot\mathbf{D}^{\mathbf{s_i}} && (78)
\end{aligned}$$

The equations central to control analysis derived above (the boxed equations) are now summarised in the normalised format:

Matrix definition of concentration-control coefficients

$$\mathbf{C^s} = -\mathcal{L}\mathcal{M}^{-1}\mathcal{N}_\mathbf{R} \tag{79}$$

Matrix definition of flux-control coefficients

$$\mathbf{C^J} = \boldsymbol{\varepsilon}_\mathbf{s}\mathbf{C^s} + \mathbf{I}_n \tag{80}$$

Partitioned concentration-response property with respect to parameters **p**:

$$\mathbf{R^s_p} = \mathbf{C^s}\boldsymbol{\varepsilon}_\mathbf{p} \tag{81}$$

Partitioned flux-response property with respect to parameters **p**:

$$\mathbf{R^J_p} = \mathbf{C^J}\boldsymbol{\varepsilon}_\mathbf{p} \tag{82}$$

The partitioned response properties with respect to **T** differ slightly from the non-normalised eqns. 59 and 64 in that the identity submatrix in the righthand matrix is replaced by a matrix containing inverse mole fractions of the dependent metabolites on its diagonal. The partitioned concentration-response property with respect to **T** is:

$$\mathbf{R^s_T} = (\mathbf{C^s}\boldsymbol{\varepsilon}_\mathbf{s} + \mathbf{I}_m)\begin{bmatrix} \mathbf{0} \\ \mathbf{D}^{\frac{\mathbf{T}}{\mathbf{s_d}}} \end{bmatrix} \tag{83}$$

Partitioned flux-response property with respect to **T**:

$$\mathbf{R^J_T} = \mathbf{C^J}\boldsymbol{\varepsilon}_\mathbf{s}\begin{bmatrix} \mathbf{0} \\ \mathbf{D}^{\frac{\mathbf{T}}{\mathbf{s_d}}} \end{bmatrix} \tag{84}$$

Relationship between $\mathbf{R^J_T}$ and $\mathbf{R^s_T}$:

$$\mathbf{R^J_T} = \boldsymbol{\varepsilon}_\mathbf{s}\mathbf{R^s_T} \tag{85}$$

4.7 Summation theorems

The summation equations for flux and concentration control coefficients follow directly from the definitions of $\mathbf{C^s}$ (eqn. 79) and $\mathbf{C^J}$ (eqn. 80) and the relationship $\mathcal{N}_\mathbf{R}\mathcal{K} = \mathbf{0}$ (the normalised form of $\mathbf{N_R K} = \mathbf{0}$). The first is called the *summation theorem for concentration-control coefficients*:

$$\mathbf{C^s}\mathcal{K} = -\mathcal{L}(\mathcal{N}_\mathbf{R}\boldsymbol{\varepsilon}_\mathbf{s}\mathcal{L})^{-1}\mathcal{N}_\mathbf{R}\mathcal{K} = \mathbf{0} \tag{86}$$

and the second the *summation theorem for flux-control coefficients*:

$$\mathbf{C^J}\mathcal{K} = (\boldsymbol{\varepsilon}_\mathbf{s}\mathbf{C^s} + \mathbf{I}_n)\mathcal{K} = \mathcal{K} \tag{87}$$

4.8 Connectivity theorems

The flux and concentration connectivity equations follows from the invertibility of the Jacobian matrix $\mathcal{N}_\mathbf{R}\boldsymbol{\varepsilon}_\mathbf{s}\mathcal{L}$ (the normalised form of $\mathbf{N_R}\bar{\boldsymbol{\varepsilon}}_\mathbf{s}\mathbf{L}$). Multiplying $\mathbf{C^s}$ and $\mathbf{C^J}$ by $\boldsymbol{\varepsilon}_\mathbf{s}\mathcal{L}$ gives, first, the *connectivity theorem for flux-control coefficients*:

$$\mathbf{C^s}\boldsymbol{\varepsilon}_\mathbf{s}\mathcal{L} = -\mathcal{L}(\mathcal{N}_\mathbf{R}\boldsymbol{\varepsilon}_\mathbf{s}\mathcal{L})^{-1}\mathcal{N}_\mathbf{R}\boldsymbol{\varepsilon}_\mathbf{s}\mathcal{L} = -\mathcal{L} \tag{88}$$

and, second, the *connectivity theorem for flux-control coefficients*:

$$\mathbf{C^J}\boldsymbol{\varepsilon}_\mathbf{s}\mathcal{L} = (\boldsymbol{\varepsilon}_\mathbf{s}\mathbf{C^s} + \mathbf{I}_n)\boldsymbol{\varepsilon}_\mathbf{s}\mathcal{L} = \mathbf{0} \tag{89}$$

Together, the summation and connectivity theorems allows the expression of control coefficients in terms of elasticity coefficients. This is arguably the most powerful feature of metabolic control analysis and is treated next.

4.9 The control-matrix equation

It is possible to combine the summation and connectivity theorems into a generalised matrix form, which we call the *control-matrix equation*. Quite a few permutations of such an equation have been suggested [2, 3, 6, 7, 8, 23, 30, 33, 34, 36, 39, 40], but the one that follows arises naturally from the formalism developed in this paper [16, 18]. Furthermore, it simplifies to the form $\mathbf{C^iE} = \mathbf{I}$ (see below), which shows explicitly how the matrix expressing independent systemic properties, $\mathbf{C^i}$, and the matrix expressing structural and local properties, **E**, are inverses of each other (if the product of two square matrices equals the identity matrix, then they are inverses of each other). This means that control coefficients can be calculated from elasticity coefficients, $\mathbf{C^i} = \mathbf{E}^{-1}$, and vice versa, $\mathbf{E} = (\mathbf{C^i})^{-1}$ (the last case being strictly true only if there are no conservation equations; see next section). The result will once again stress the fundamental role of the $\mathcal{K}$ and $\mathcal{L}$-matrices in control analysis.

The control-matrix equation is formed by combining eqns. 86–89 as [16, 18]:

$$\begin{bmatrix} \mathbf{C^J} \\ \mathbf{C^s} \end{bmatrix}\begin{bmatrix} \mathcal{K} & -\boldsymbol{\varepsilon}_\mathbf{s}\mathcal{L} \end{bmatrix} = \begin{bmatrix} \mathcal{K} & \mathbf{0} \\ \mathbf{0} & \mathcal{L} \end{bmatrix} \tag{90}$$

The matrices can be partitioned in terms of independent and dependent variables to give

$$\begin{bmatrix} \mathbf{C^{J_i}} \\ \mathbf{C^{J_d}} \\ \mathbf{C^{s_i}} \\ \mathbf{C^{s_d}} \end{bmatrix}\begin{bmatrix} \mathcal{K} & -\boldsymbol{\varepsilon}_\mathbf{s}\mathcal{L} \end{bmatrix} = \begin{bmatrix} \mathbf{I}_{n-r} & \mathbf{0} \\ \mathcal{K}_0 & \mathbf{0} \\ \mathbf{0} & \mathbf{I}_r \\ \mathbf{0} & \mathcal{L}_\mathbf{0} \end{bmatrix} \tag{91}$$

Extracting the equations for the independent variables $\mathbf{J_i}$ and $\mathbf{s_i}$ gives:

$$\begin{bmatrix} \mathbf{C^{J_i}} \\ \mathbf{C^{s_i}} \end{bmatrix}\begin{bmatrix} \mathcal{K} & -\boldsymbol{\varepsilon}_\mathbf{s}\mathcal{L} \end{bmatrix} = \begin{bmatrix} \mathbf{I}_{n-r} & \mathbf{0} \\ \mathbf{0} & \mathbf{I}_r \end{bmatrix} \tag{92}$$

which, if $\mathbf{C^i} = [\mathbf{C^{J_i}}\ \mathbf{C^{s_i}}]^T$ and $\mathbf{E} = [\mathcal{K}\ -\boldsymbol{\varepsilon}_\mathbf{s}\mathcal{L}]$, reduces to the particularly elegant form:

$$\mathbf{C^iE} = \mathbf{I}_n \tag{93}$$

Both $\mathbf{C^i}$ and **E** are square invertible $n \times n$ matrices [13, 16], i.e., the equation can also be written as $\mathbf{EC^i} = \mathbf{I}$, which expresses flux-control coefficients in terms of concentration-control and elasticity coefficients. These equations are completely general and hold for any network of reactions.

4.10 The inverse problem

We have seen that $\mathbf{C^i} = \mathbf{E}^{-1}$: if all the elasticity coefficients have been determined (either experimentally or by calculation as the normalised partial derivatives of the rate equations), the control coefficients with respect to the independent concentrations and fluxes can be calculated by inverting **E**. The control coefficients with respect to the dependent variables are calculated using the relationships:

$$\mathbf{C^{s_d}} = \mathcal{L}_\mathbf{0}\mathbf{C^{s_i}} \tag{94}$$

$$\mathbf{C^{J_d}} = \mathcal{K}_0\mathbf{C^{J_i}} \tag{95}$$

which follow from eqns. 2 and 18.

However, consider the inverse problem, i.e., calculating the elasticity coefficients from experimentally determined control coefficients. Using $\mathbf{E} = (\mathbf{C^i})^{-1}$ we can calculate **E** by inverting $\mathbf{C^i}$. If $\mathcal{L} = \mathbf{I}$, i.e., if there are no conservation constraints, the task is accomplished—the righthand r columns of **E** form the elasticity matrix $-\boldsymbol{\varepsilon}_\mathbf{s}$ and therefore contain the values of the elasticity coefficients. However, if $\mathcal{L} \neq \mathbf{I}$, some elements in the righthand r columns of **E** contain linear functions of elasticity coefficients, and more information is needed to solve for the individual elasticities.

This extra information can only be obtained by perturbing the conservations sums in the column vector $\mathbf{T}$ and measuring the resulting steady-state changes in *all* the fluxes and concentrations. We augment on the left both sides of eqn. 85:

$$\mathbf{R}^{\mathbf{J}}_{\mathbf{T}} = \boldsymbol{\varepsilon}_{\mathbf{s}}\mathbf{R}^{\mathbf{s}}_{\mathbf{T}} \tag{96}$$

with the matrix $\boldsymbol{\varepsilon}_{\mathbf{s}}\boldsymbol{\mathcal{L}}$ to give

$$[\boldsymbol{\varepsilon}_{\mathbf{s}}\boldsymbol{\mathcal{L}} \;\; \mathbf{R}^{\mathbf{J}}_{\mathbf{T}}] = [\boldsymbol{\varepsilon}_{\mathbf{s}}\boldsymbol{\mathcal{L}} \;\; \boldsymbol{\varepsilon}_{\mathbf{s}}\mathbf{R}^{\mathbf{s}}_{\mathbf{T}}] \tag{97}$$

which can be re-arranged to solve for $\boldsymbol{\varepsilon}_{\mathbf{s}}$:

$$\boldsymbol{\varepsilon}_{\mathbf{s}} = [\boldsymbol{\varepsilon}_{\mathbf{s}}\boldsymbol{\mathcal{L}} \;\; \mathbf{R}^{\mathbf{J}}_{\mathbf{T}}][\boldsymbol{\mathcal{L}} \;\; \mathbf{R}^{\mathbf{s}}_{\mathbf{T}}]^{-1} \tag{98}$$

The $n \times n$ matrix $[\boldsymbol{\mathcal{L}} \;\; \mathbf{R}^{\mathbf{s}}_{\mathbf{T}}]$ has been proved to be invertible [1].

5. DISCUSSION

This paper set out to provide, in a nutshell, the complete formal basis for metabolic control analysis in a way that leaves as little as possible unexplained. In particular, care has been taken to show how the functional relationships in the steady-state equations hang together, thereby proscribing how the steady-state equations should be differentiated. For a more extensive exposition of much of the material covered in this paper the reader is referred to the excellent monograph by Heinrich and Schuster [13], which is a treasure trove of information on biochemical modelling and control analysis. However, there are aspects covered here which are either absent from their treatment (the response to $\mathbf{T}$ in Sections 4.4 and 4.5, and the inverse problem in 4.10) or different (Sections 4.7, 4.8, and 4.9, where full scaling is used instead of the partial scaling used in [13]).

Metabolic control analysis has been applied to many types of systems, which has led to interesting extensions of the theory, for example, multi-level or hierarchical systems [20, 24], modular systems [31, 35], signal transduction pathways [26], time-dependent phenomena [12, 13], transition times [28], oscillating systems [4], channelled systems [25], and group-transfer pathways [27].

Co-response analysis [16] is an extension built on the control-matrix equation described in this paper. It not only has useful experimental implications (control analysis requiring neither kinetic knowledge of the component reactions nor quantitative information about the magnitudes of the effects of perturbations on individual enzyme activities), but also forms the basis for the analysis of regulatory aspects of metabolism (for example, supply-demand analysis [14, 15, 17]).

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APPENDIX
A. THE JACOBIAN MATRIX

In general, any dynamical system $d\mathbf{x}/dt = \mathbf{f}(\mathbf{x})$, where the $\mathbf{f}$ are nonlinear functions of $\mathbf{x}$, can be linearised around any current state $\mathbf{x}^o$ (transient or steady) to give $d\mathbf{x}^o/dt = \mathbf{M}\mathbf{x}^o$, where $\mathbf{M}$ is the Jacobian matrix $\partial\mathbf{f}/\partial\mathbf{x}$, i.e., the matrix of partial derivatives of $\mathbf{f}$ with respect to $\mathbf{x}$ evaluated at state $\mathbf{x}^o$. If a steady state is considered, a necessary condition for its existence is that the Jacobian matrix be invertible (its determinant be non-zero), and for its asymptotic stability that the eigenvalues of the Jacobian matrix have negative real parts (see, e.g., [13]). The Jacobian matrix therefore characterises the local behaviour around the steady state. We now show that for the kinetic model discussed in this paper $\mathbf{M} = \mathbf{N_R}\bar{\boldsymbol{\varepsilon}}_{\mathbf{s}^o}\mathbf{L}$ is the Jacobian matrix.

Assume that for the kinetic model

$$\frac{d\mathbf{s}}{dt} = \mathbf{N}\mathbf{v}[\mathbf{s}, \mathbf{p}] \tag{99}$$

the current state is symbolised by the vector of concentrations $\mathbf{s}^o$. As $\mathbf{p}$ is assumed to be constant in the following, we simplify the representation of the kinetic model to

$$\frac{d\mathbf{s}^o}{dt} = \mathbf{N}\mathbf{v}[\mathbf{s}^o] \tag{100}$$

When the current concentrations are perturbed by $\delta\mathbf{s}$ so that

$$\mathbf{s}(t) = \mathbf{s}^o + \delta\mathbf{s}(t) \tag{101}$$

the kinetic model becomes:

$$\frac{d}{dt}(\mathbf{s}^o + \delta\mathbf{s}) = \mathbf{N}\mathbf{v}[\mathbf{s}^o + \delta\mathbf{s}] \tag{102}$$

From multivariate calculus we know that Taylor's theorem enables us to approximate to any degree of accuracy the function $\mathbf{v}[\mathbf{s}^o + \delta\mathbf{s}]$ by the expansion

$$\mathbf{v}[\mathbf{s}^o] + \left(\frac{\partial\mathbf{v}}{\partial\mathbf{s}}\right)_{\mathbf{s}^o}(\delta\mathbf{s}) + \frac{1}{2!}\left(\frac{\partial^2\mathbf{v}}{\partial\mathbf{s}^2}\right)_{\mathbf{s}^o}(\delta\mathbf{s})^2 + \text{higher order terms} \tag{103}$$

For small deviations $\delta\mathbf{s}$ the first two terms suffice to approximate the function, so that

$$\frac{d\mathbf{s}^o}{dt} + \frac{d}{dt}(\delta\mathbf{s}) = \mathbf{N}\mathbf{v}[\mathbf{s}^o] + \mathbf{N}\left(\frac{\partial\mathbf{v}}{\partial\mathbf{s}}\right)_{\mathbf{s}^o}(\delta\mathbf{s}) \tag{104}$$

which amounts to a linearisation around state $\mathbf{s}^o$. By definition the matrix of partial derivatives $\partial\mathbf{v}/\partial\mathbf{s}$ is the matrix of non-normalised elasticity coefficients $\bar{\boldsymbol{\varepsilon}}_{\mathbf{s}^o}$ evaluated at the state characterised by $\mathbf{s}^o$. Using eqn. 100 we obtain the linearised form of the kinetic model at state $\mathbf{s}^o$:

$$\frac{d}{dt}(\delta\mathbf{s}) = \mathbf{N}\bar{\boldsymbol{\varepsilon}}_{\mathbf{s}^o} \cdot \delta\mathbf{s} \tag{105}$$

If the number of independent concentrations is less than the number of metabolites then by definition $\mathbf{N} = \mathbf{L}\mathbf{N}_\mathbf{R}$ and $\mathbf{s} = \mathbf{L}\mathbf{s}_\mathbf{i}$, or equivalently, $\delta\mathbf{s} = \mathbf{L}\delta\mathbf{s}_\mathbf{i}$. Using the argument of eqns. 4–6 it follows that

$$\frac{d}{dt}(\delta\mathbf{s}_\mathbf{i}) = \mathbf{N}_\mathbf{R}\bar{\boldsymbol{\varepsilon}}_{\mathbf{s}^o}\mathbf{L}\cdot\delta\mathbf{s}_\mathbf{i} \tag{106}$$

From the general definition of the Jacobian matrix given in the first paragraph of this section we see that

$$\mathbf{M} = \mathbf{N}_\mathbf{R}\bar{\boldsymbol{\varepsilon}}_{\mathbf{s}^o}\mathbf{L} \tag{107}$$

is the Jacobian matrix.

The Jacobian matrix can be normalised as follows: Define the diagonal matrices $\mathbf{D}^{\mathbf{v}^o}$, $\mathbf{D}^{\mathbf{s}^o}$ and $\mathbf{D}^{\mathbf{s}^o_\mathbf{i}}$ which respectively have the reaction rates, concentrations and independent concentrations obtaining at state $\mathbf{s}^o$ on their diagonal (the rate and concentration vectors are arranged so that the independent variables come first, the dependent variables second). Their inverses $(\mathbf{D}^{\mathbf{v}^o})^{-1}$, $(\mathbf{D}^{\mathbf{s}^o})^{-1}$ and $(\mathbf{D}^{\mathbf{s}^o_\mathbf{i}})^{-1}$ have the inverse rates and inverse concentrations on their diagonals.

Using the identities

$$\delta\ln\mathbf{s}_\mathbf{i} = (\mathbf{D}^{\mathbf{s}^o_\mathbf{i}})^{-1}\delta\mathbf{s}_\mathbf{i} \tag{108}$$

$$\boldsymbol{\mathcal{N}}_\mathbf{R} = (\mathbf{D}^{\mathbf{s}^o_\mathbf{i}})^{-1}\cdot\mathbf{N}_\mathbf{R}\cdot\mathbf{D}^{\mathbf{v}^o} \tag{109}$$

$$\boldsymbol{\varepsilon}_{\mathbf{s}^o} = (\mathbf{D}^{\mathbf{v}^o})^{-1}\cdot\bar{\boldsymbol{\varepsilon}}_{\mathbf{s}^o}\cdot\mathbf{D}^{\mathbf{s}^o} \tag{110}$$

$$\boldsymbol{\mathcal{L}} = (\mathbf{D}^{\mathbf{s}^o})^{-1}\cdot\mathbf{L}\cdot\mathbf{D}^{\mathbf{s}^o_\mathbf{i}} \tag{111}$$

eqn. 106 can be written as

$$\frac{d}{dt}(\delta\ln\mathbf{s}_\mathbf{i}) = \boldsymbol{\mathcal{N}}_\mathbf{R}\boldsymbol{\varepsilon}_{\mathbf{s}^o}\boldsymbol{\mathcal{L}}\cdot\delta\ln\mathbf{s}_\mathbf{i} \tag{112}$$

The kinetic model for perturbations from state $\mathbf{s}^o$ has therefore been transformed to logarithmic space. From this formulation the normalised Jacobian matrix is seen to be

$$\boldsymbol{\mathcal{M}} = \boldsymbol{\mathcal{N}}_\mathbf{R}\boldsymbol{\varepsilon}_{\mathbf{s}^o}\boldsymbol{\mathcal{L}} \tag{113}$$

which, if there are no dependent metabolites ($\boldsymbol{\mathcal{L}} = \mathbf{I}$) simplifies to

$$\boldsymbol{\mathcal{M}} = \boldsymbol{\mathcal{N}}\boldsymbol{\varepsilon}_{\mathbf{s}^o} \tag{114}$$

B. AN EXPLICIT EXAMPLE

Fig. 1 represents a simple reaction network containing both a branched flux and a moiety-conserved cycle [19]. Here we show how the **K** and **L**-matrices can be constructed from an analysis of its stoichiometric matrix. Once these matrices are available it is a simple matter to formulate the control matrix equation $\mathbf{C}^\mathbf{i}\mathbf{E} = \mathbf{I}$ explicitly (for a numerical solution of this example see [16]).

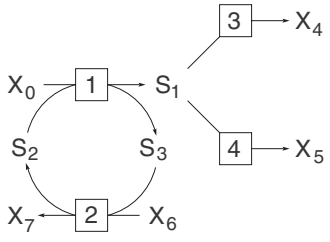

Figure 1: A example reaction network.

The first step is to write down the stoichiometric matrix **N** for this system, labelling the rows and columns (the left-hand matrix in eqn. 115). **N** is then augmented with an identity matrix in which each column represents a time derivative (the right-hand matrix in eqn. 115). Note that only variable metabolites S_i are represented. The terminal X-metabolite concentrations must be constant (at non-equilibrium values) for a steady state to exist, and are therefore considered part of the parameter set.

	R_1	R_2	R_3	R_4	$\dot{s}_1$	$\dot{s}_2$	$\dot{s}_3$
S_1	1	0	−1	−1	1	0	0
S_2	−1	1	0	0	0	1	0
S_3	1	−1	0	0	0	0	1

(115)

Next the augmented matrix is subjected to Gaussian elimination to row echelon form[1]:

	R_1	R_2	R_3	R_4	$\dot{s}_1$	$\dot{s}_2$	$\dot{s}_3$
S_1	1	0	−1	−1	1	0	0
S_2	0	1	−1	−1	1	1	0
S_3	0	0	0	0	0	1	1

(116)

The rank of the stoichiometric matrix is 2 and there is one conservation relationship $s_2 + s_3 = T$, where T is the conserved sum. There are two independent fluxes and two independent metabolites.

Choosing J_3 and J_4 (the R_3 and R_4-columns without pivots in the reduced stoichiometric matrix $\mathbf{N}_\mathbf{R}$ in eqn. 116) as the independent fluxes, the **K**-matrix follows from the flux relationships $\mathbf{J} = \mathbf{K}\mathbf{J}_\mathbf{i}$:

$$\begin{bmatrix} J_3 \\ J_4 \\ J_1 \\ J_2 \end{bmatrix} = \begin{bmatrix} J_3 \\ J_4 \\ J_3 + J_4 \\ J_3 + J_4 \end{bmatrix} = \begin{bmatrix} 1 \\ 0 \\ 1 \\ 1 \end{bmatrix} J_3 + \begin{bmatrix} 0 \\ 1 \\ 1 \\ 1 \end{bmatrix} J_4 = \begin{bmatrix} 1 & 0 \\ 0 & 1 \\ 1 & 1 \\ 1 & 1 \end{bmatrix} \begin{bmatrix} J_3 \\ J_4 \end{bmatrix} \tag{117}$$

K is scaled to $\mathcal{K} = (\mathbf{D}^\mathbf{J})^{-1}\mathbf{K}\mathbf{D}^{\mathbf{J}_\mathbf{i}}$ as follows:

$$\begin{bmatrix} \frac{1}{J_3} & 0 & 0 & 0 \\ 0 & \frac{1}{J_4} & 0 & 0 \\ 0 & 0 & \frac{1}{J_1} & 0 \\ 0 & 0 & 0 & \frac{1}{J_2} \end{bmatrix} \begin{bmatrix} 1 & 0 \\ 0 & 1 \\ 1 & 1 \\ 1 & 1 \end{bmatrix} \begin{bmatrix} J_3 & 0 \\ 0 & J_4 \end{bmatrix} = \begin{bmatrix} 1 & 0 \\ 0 & 1 \\ \frac{J_3}{J_1} & \frac{J_4}{J_1} \\ \frac{J_3}{J_2} & \frac{J_4}{J_2} \end{bmatrix} \tag{118}$$

Either S_2 or S_3 can be chosen as the dependent metabolite. We choose S_3. The **L**-matrix follows from the relationships in $d\mathbf{s}/dt = \mathbf{L}d\mathbf{s}_\mathbf{i}/dt$, which are read off from the last row of the righthand matrix in eqn. 116:

$$\begin{bmatrix} \dot{s}_1 \\ \dot{s}_2 \\ \dot{s}_3 \end{bmatrix} = \begin{bmatrix} \dot{s}_1 \\ \dot{s}_2 \\ -\dot{s}_2 \end{bmatrix} = \begin{bmatrix} 1 \\ 0 \\ 0 \end{bmatrix} \dot{s}_1 + \begin{bmatrix} 0 \\ 1 \\ -1 \end{bmatrix} \dot{s}_2 = \begin{bmatrix} 1 & 0 \\ 0 & 1 \\ 0 & -1 \end{bmatrix} \begin{bmatrix} \dot{s}_1 \\ \dot{s}_2 \end{bmatrix} \tag{119}$$

L is scaled to $\boldsymbol{\mathcal{L}} = (\mathbf{D}^\mathbf{s})^{-1}\mathbf{L}\mathbf{D}^{\mathbf{s}_\mathbf{i}}$ as follows:

$$\begin{bmatrix} \frac{1}{s_1} & 0 & 0 \\ 0 & \frac{1}{s_2} & 0 \\ 0 & 0 & \frac{1}{s_3} \end{bmatrix} \begin{bmatrix} 1 & 0 \\ 0 & 1 \\ 0 & -1 \end{bmatrix} \begin{bmatrix} s_1 & 0 \\ 0 & s_2 \end{bmatrix} = \begin{bmatrix} 1 & 0 \\ 0 & 1 \\ 0 & -\frac{s_2}{s_3} \end{bmatrix} \tag{120}$$

The matrix product $-\boldsymbol{\varepsilon}_\mathbf{s}\boldsymbol{\mathcal{L}}$ for this system is

$$-\begin{bmatrix} \varepsilon_{s_1}^{v_3} & 0 & 0 \\ \varepsilon_{s_1}^{v_4} & 0 & 0 \\ \varepsilon_{s_1}^{v_1} & \varepsilon_{s_2}^{v_1} & \varepsilon_{s_3}^{v_1} \\ 0 & \varepsilon_{s_2}^{v_2} & \varepsilon_{s_3}^{v_2} \end{bmatrix} \begin{bmatrix} 1 & 0 \\ 0 & 1 \\ 0 & -\frac{s_2}{s_3} \end{bmatrix} = \begin{bmatrix} -\varepsilon_{s_1}^{v_3} & 0 \\ -\varepsilon_{s_1}^{v_4} & 0 \\ -\varepsilon_{s_1}^{v_1} & (\varepsilon_{s_3}^{v_1}\frac{s_2}{s_3} - \varepsilon_{s_2}^{v_1}) \\ 0 & (\varepsilon_{s_3}^{v_2}\frac{s_2}{s_3} - \varepsilon_{s_2}^{v_2}) \end{bmatrix} \tag{121}$$

[1]Simple systems can be analysed by hand, more complicated systems with one of the numerous computer tools that do this automatically, such as Mathematica, Matlab, etc. or dedicated metabolic simulators (e.g., [29, 32, 38], Metatool [ftp://mudshark.brookes.ac.uk/pub/software/ibmpc/metatool])

Note that the re-ordering of fluxes in the $\mathcal{K}$-matrix is reflected in $\boldsymbol{\varepsilon}_\mathbf{s}$.

Finally, the $\mathbf{C^i E} = \mathbf{I}$ control-matrix equation is constructed using $\mathcal{K}$ and $-\boldsymbol{\varepsilon}_\mathbf{s}\mathcal{L}$:

$$\begin{bmatrix} C_3^{J_3} & C_4^{J_3} & C_1^{J_3} & C_2^{J_3} \\ C_3^{J_4} & C_4^{J_4} & C_1^{J_4} & C_2^{J_4} \\ C_3^{s_1} & C_4^{s_1} & C_1^{s_1} & C_2^{s_1} \\ C_3^{s_2} & C_4^{s_2} & C_1^{s_2} & C_2^{s_2} \end{bmatrix} \times \begin{bmatrix} 1 & 0 & -\varepsilon_{s_1}^{v_3} & 0 \\ 0 & 1 & -\varepsilon_{s_1}^{v_4} & 0 \\ \frac{J_3}{J_1} & \frac{J_4}{J_1} & -\varepsilon_{s_1}^{v_1} & (\varepsilon_{s_3}^{v_1}\frac{s_2}{s_3} - \varepsilon_{s_2}^{v_1}) \\ \frac{J_3}{J_2} & \frac{J_4}{J_2} & 0 & (\varepsilon_{s_3}^{v_2}\frac{s_2}{s_3} - \varepsilon_{s_2}^{v_2}) \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix} \tag{122}$$

To solve the inverse problem ($\boldsymbol{\varepsilon}_\mathbf{s}$ from $(\mathbf{C^i})^{-1}$,$\mathbf{R_T^s}$, and $\mathbf{R_T^J}$) we need to construct eqn. 98. There is only one conserved sum $s_2 + s_3 = T$. The matrix product $\boldsymbol{\varepsilon}_\mathbf{s}\mathcal{L}$ is known from $(\mathbf{C^i})^{-1}$, so that

$$\boldsymbol{\varepsilon}_\mathbf{s} = \begin{bmatrix} \varepsilon_{s_1}^{v_3} & 0 & R_T^{J_3} \\ \varepsilon_{s_1}^{v_4} & 0 & R_T^{J_4} \\ \varepsilon_{s_1}^{v_1} & (\varepsilon_{s_2}^{v_1} - \varepsilon_{s_3}^{v_1}\frac{s_2}{s_3}) & R_T^{J_1} \\ 0 & (\varepsilon_{s_2}^{v_2} - \varepsilon_{s_3}^{v_2}\frac{s_2}{s_3}) & R_T^{J_2} \end{bmatrix} \begin{bmatrix} 1 & 0 & R_T^{s_1} \\ 0 & 1 & R_T^{s_2} \\ 0 & -\frac{s_2}{s_3} & R_T^{s_3} \end{bmatrix}^{-1} \tag{123}$$