# Sensitivity analysis of autonomous oscillations: application to biochemical systems

Brian P. Ingalls
Department of Applied Mathematics
University of Waterloo
Waterloo, Ontario
Canada N2L 3G1
bingalls@math.uwaterloo.ca

## Abstract

This work addresses sensitivity analysis of autonomously oscillating biochemical systems by extending previous treatments which considered special cases or empirical approximations to system response. Building on results from the engineering literature, an analysis is presented which addresses sensitivity of key features of the oscillatory trajectory: namely the period and local maximum or minimum values of species concentrations and reaction rates. A discussion of sensitivity invariants generalizes results from steady-state sensitivity analysis to this context.

## 1 Introduction

Sensitivity analysis plays an important role in the study of biochemical systems. Mathematical approaches to such analysis have been introduced through the fields of Metabolic Control Analysis (MCA) [11, 6] and Biochemical Systems Theory (BST) [18]. These analyses have proven useful both by providing insight into the nature and function of biochemical systems as well as by serving as a tool to predict the result of interventions, e.g. for metabolic engineering or pharmaceutic purposes.

In the study of biochemical networks, it is often the case that steady state behaviour is of primary interest (not least because this is the simplest behaviour to investigate experimentally). As a consequence, the sensitivity analysis of systems at steady state has proven adequate for many investigations.

However, the dynamic behaviour of biochemical systems is increasingly coming under investiga-

tion. This is due in part to advances in experimental techniques which make observation of time series more readily attainable, and in part to an increasing awareness that a complete understanding of many of the mechanisms within the cell will only be reached once their dynamic behaviour has been described. As a result, a number of approaches to the extension of classical (steady-state) sensitivity analysis have appeared.

General definitions of time-varying sensitivity functions were first given in this context in [1] (see also [10, 12]). This extension of the standard analysis allows treatment of systems whose behaviour is primarily dynamic. While these results have proven to be useful, there is one form of dynamic behaviour for which they cannot provide satisfactory descriptions, namely autonomously oscillating systems. Such system underly many of the periodic phenomena which have been identified in biology (e.g. glycolytic oscillations, the cell cycle, circadian rhythms, periodic neuronal signals). See [7] for a review.

It has long been recognized ([2] and references therein) that the sensitivity of various features of oscillating systems (e.g. period and amplitude) could be estimated experimentally (or through simulation), even if there was no general theory to provide a means of analytic computation. A first step towards such a theory was presented in [13], in which a satisfactory treatment of systems exhibiting *forced* oscillations is given. This theory was complemented by the results in [4] which address sensitivity of Fourier coefficients. Autonomously oscillating systems were treated by this approach in [16]. These works provide vital contributions to the study of periodic systems. However, there has yet to appear a general treatment of sensitivity of autonomously oscillating

biochemical systems. This paper complements the existing work by providing such an exposition.

Sensitivity analysis is a crucial aspect of the study of control systems, and has been extensively treated within the engineering community. In [3], Buré and Rosenvasser present a method for deriving sensitivity of features of limit cycle trajectories (namely period and amplitude) to changes in parameters. The same results were derived independently in [5, 14]. As the current paper will demonstrate, this method can be readily applied to biochemical systems and the resulting analytic description can be used to provide additional insight in this setting.

## 2 Preliminaries

The analysis will treat a general network of $n$ chemical species ($\mathbf{s} = (s_1, s_2, \ldots, s_n)$) involved in $m$ reactions in a fixed volume. The system will be modelled as depending on a single scalar parameter $p$. The system is described by the $n$ by $m$ stoichiometry matrix $\mathbf{N}$ and the $m$-vector valued reaction rate function $\mathbf{v} = \mathbf{v}(\mathbf{s}, p)$. The dynamics are given by

$$\frac{d}{dt}\mathbf{s}(t) = \mathbf{N}\mathbf{v}(\mathbf{s}(t), p) \qquad \text{for all } t \geq 0. \qquad (1)$$

The parameter $p$ might represent any of the parameters of the model (e.g. a $V_{\max}$ or $K_m$ value) or any particular direction in the parameter space. The function $\mathbf{v}$ is assumed continuously differentiable.

Stoichiometric systems may contain redundant states (e.g. due to conserved moieties), which correspond to linear dependencies among the rows of $\mathbf{N}$. In such cases, the number of states can be reduced for the purposes of analysis and computation, as discussed in [15]. In order to simplify the presentation in what follows, we will assume that no such reduction is called for.

In what follows we will assume that for each value of the parameter $p$ in the range of interest, the system (1) exhibits a periodic trajectory $\mathbf{s}_{\mathrm{per}}(t, p)$, with period $T(p)$, i.e.

$$\mathbf{s}_{\mathrm{per}}(t + T(p), p) = \mathbf{s}_{\mathrm{per}}(t, p) \qquad \forall t \geq 0. \qquad (2)$$

Moreover, it will be assumed that each such trajectory is a stable limit cycle.

## 3 Sensitivity Analysis

As described in [1] and [12], the general (time-varying) *concentration response coefficient* (or concentration sensitivity function) is defined as follows.

**Definition 3.1** Given a nominal parameter value $p_0$ and a corresponding periodic trajectory $\mathbf{s}_{\mathrm{per}}(\cdot, p_0)$, define the *response coefficient* as the $n$-vector valued function $\mathbf{R}^{\mathbf{s}}(\cdot)$ given by

$$\mathbf{R}^{\mathbf{s}}(t) := \frac{\partial \mathbf{s}_{\mathrm{per}}(t, p)}{\partial p}\Big|_{p=p_0} \qquad \forall t \geq 0. \qquad (3)$$

The evolution of this sensitivity function is described by the first order linear differential equation

$$\frac{d}{dt}\mathbf{R}^{\mathbf{s}}(t) = \mathbf{N}\frac{\partial \mathbf{v}(t)}{\partial \mathbf{s}}\mathbf{R}^{\mathbf{s}}(t) + \mathbf{N}\frac{\partial \mathbf{v}(t)}{\partial p} \quad \forall t \geq 0. \quad (4)$$

The partial derivatives $\frac{\partial \mathbf{v}(t)}{\partial \mathbf{s}}$ and $\frac{\partial \mathbf{v}(t)}{\partial p}$ are evaluated along the trajectory $\mathbf{s}_{\mathrm{per}}(t, p_0)$.

The response of system (1) to perturbations in parameters which affect only the initial conditions is straightforward, as was discussed in [12]. Henceforth we will assume that a change in the parameter $p$ has no effect at time $t = 0$, and so we will take initial condition $\mathbf{R}^{\mathbf{s}}(0) = \mathbf{0}$ in equation (4).

In standard sensitivity analysis, the response coefficients are used directly to determine the system behaviour under perturbations. However, as discussed in [13], the sensitivity functions for autonomously oscillating systems grow unbounded in time and hence are of little direct use in addressing asymptotic behaviour. Nonetheless, as described in [3], these sensitivity functions can be use to describe the asymptotic response of certain properties of the oscillations, as outlined below.

Before addressing sensitivity, we make a remark regarding equation (4). Observe that when evaluated along the periodic trajectory $\mathbf{s}_{\mathrm{per}}(t, p)$ both $\mathbf{N}\frac{\partial \mathbf{v}(t)}{\partial \mathbf{s}}$ and $\mathbf{N}\frac{\partial \mathbf{v}(t)}{\partial p}$ are $T(p)$-periodic. The homogeneous part of equation (4), whose fundamental matrix $\mathbf{H}(\cdot)$ is the solution of

$$\frac{d}{dt}\mathbf{H}(t) = \mathbf{N}\frac{\partial \mathbf{v}(t)}{\partial \mathbf{s}}\mathbf{H}(t) \qquad \mathbf{H}(0) = \mathbf{I}, \qquad (5)$$

can thus be addressed by the tools of Floquet Theory (see, e.g. [8]). The result is a solution of the form

$$\mathbf{H}(t) = \mathbf{D}(t)e^{\mathbf{B}t} \qquad (6)$$

where the matrix $\mathbf{D}(\cdot)$ is $T(p)$-periodic. The eigenvalues of the matrix $\mathbf{B}$ are called the *characteristic exponents* of the system. Under our assumption that the system (1) admits the $T(p)$-periodic solution $\mathbf{s}_{\mathrm{per}}(\cdot, p)$, it follows that the differential equation in (5) likewise admits a $T(p)$-periodic solution. Comparing with (6), one concludes that the matrix $\mathbf{B}$ must have at least one eigenvalue with modulus one.

We now make the assumption that all of the other characteristic exponents are in the unit circle, which is consistent with the fact that the periodic trajectory $\mathbf{s}_{\mathrm{per}}(\cdot, p)$ is a limit cycle. Under this assumption, the fundamental matrix $\mathbf{H}(\cdot)$ can be expressed as

$$\mathbf{H}(t) = \mathbf{H}_{\mathrm{per}}(t) + \mathbf{H}_{\mathrm{trans}}(t) \qquad \forall t \geq 0,$$

where $\lim_{t\to\infty} \mathbf{H}_{\mathrm{trans}}(t) = \mathbf{0}$ and $\mathbf{H}_{\mathrm{per}}(\cdot)$ is $T(p)$-periodic.

As mentioned earlier, the unbounded nature of the sensitivity function means that it cannot be used directly to describe the asymptotic system response. However, the *variation* in the sensitivity function over the period $T(p_0)$ is a solution of equation (5), and hence is better behaved. This variation proves useful, as shown below.

### 3.1 Sensitivity of Oscillatory Characteristics

In addressing the sensitivity of autonomously oscillating systems, it is the *asymptotic* response which is of primary interest. In the case of asymptotically stable systems, the response coefficients as defined above tend to the steady-state sensitivity coefficients as time grow large. For autonomously oscillating systems, this interpretation cannot be exploited since the response coefficients diverge. However, their values do converge to a description of the asymptotic behaviour of the *extreme points* on the periodic trajectory, as shown below.

### Sensitivity of Local Extrema

The oscillatory behaviour of systems is often described in terms of local extrema, e.g. maximal or minimal levels of chemical species. The extreme levels of flux through a pathway are also of interest in addressing periodic systems.

Having identified such extrema as of interest, one can then ask how they may be affected by a parameter variation. A satisfactory answer can be given provided one characterizes the point by the *time* at which it occurs. That is, having fixed a time $t^0$ of interest on the periodic trajectory

$\mathbf{s}_{\mathrm{per}}(\cdot, p_0)$, one can describe the response of the system at time $t^0$ to a change in $p$, provided this time represents a local maximum or minimum value for the variable of interest, as we now demonstrate.

The following is a straightforward extension of results in [17]. The proof is omitted due to lack of space.

Let $Y : \mathbb{R}^n \times \mathbb{R} \to \mathbb{R}$ be some function of the state and the parameter, and identify a time $t^0(p_0) \in [0, T(p_0))$ at which the function $Y(\mathbf{s}_{\mathrm{per}}(t, p_0), p_0)$ achieves a local extremum. Then, for integers $m$,

$$\left. \frac{d}{dp} Y(\mathbf{s}_{\mathrm{per}}(t^0(p), p), p) \right|_{p=p_0} = \qquad (7)$$

$$\lim_{m\to\infty} \frac{\partial Y}{\partial \mathbf{s}} \mathbf{R}^{\mathbf{s}}(t^0(p_0) + mT(p_0)) + \frac{\partial Y}{\partial p}, (8)$$

where the partial derivatives of $Y$ are evaluated at $(\mathbf{s}_{\mathrm{per}}(t^0(p_0), p_0), p_0)$.

In addressing biochemical networks, the most common choices for the function $Y$ are specific species concentrations or reaction rates. This second choice leads to sensitivity functions known as flux response coefficients, denoted by the vector $\mathbf{R}^{\mathbf{v}}(\cdot)$. Using $\mathbf{R}^{\mathbf{s}*}$ and $\mathbf{R}^{\mathbf{v}*}$ to denote the asymptotic response of the species concentrations and reaction rates, respectively, (7) provides the following. For any species $s_i$ or reaction rate $v_j$ which achieves an extreme value at $t^0$,

$$\mathbf{R}^{s_i*}(t^0) = \lim_{m\to\infty} \mathbf{R}^{s_i}(t^0(p_0) + mT(p_0))$$

$$\mathbf{R}^{v_j*}(t^0) = \lim_{m\to\infty} \frac{\partial v_j}{\partial \mathbf{s}} \mathbf{R}(t^0(p_0) + mT(p_0)) + \frac{\partial v_j}{\partial p}$$

where the partial derivatives are evaluated at $(\mathbf{s}_{\mathrm{per}}(t^0(p_0), p_0), p_0)$.

### Sensitivity of the Period

In addition to maximal and minimal values, the asymptotic response of the oscillation can also be characterized by the change in the period. An auxiliary definition will be needed.

**Definition 3.2** Given a solution $\mathbf{R}^{\mathbf{s}}(\cdot)$ of (4), define the *variation of the sensitivity* over the period of the nominal oscillation by

$$\Delta \mathbf{R}^{\mathbf{s}}(t) = \mathbf{R}^{\mathbf{s}}(t + T(p_0)) - \mathbf{R}^{\mathbf{s}}(t), \qquad \forall t \geq 0.$$

As described in [3], assuming $T(\cdot)$ is differentiable, the sensitivity of the period can expressed as follows: for any $i = 1, 2, \ldots, n$, let $\mathbf{\Delta R}^{s_i}(t)$

denote the $i$-th element of $\mathbf{\Delta R^s}(t)$, let $\mathbf{N}_i$ be the $i$-th row of $\mathbf{N}$, and choose any unbounded increasing sequence of times $\{t_k\}_{k=1}^{\infty}$ such that $\mathbf{N}_i \mathbf{v}(\mathbf{s}_{\mathrm{per}}(t_k, p_0), p_0) \neq 0$ for all $k$. Then

$$\left. \frac{dT(p)}{dp} \right|_{p=p_0} = \lim_{k \to \infty} -\frac{\mathbf{\Delta R}^{s_i}(t_k)}{\mathbf{N}_i \mathbf{v}(\mathbf{s}_{\mathrm{per}}(t_k, p_0), p_0)}. \quad (9)$$

### 3.2 A Remark on Computation

In previous treatments, the sensitivity coefficients derived above have only been treated analytically in special cases (e.g. [1]). More commonly, they have been approximated through simulation, e.g. by making a 1% change in a parameter and observing the resulting behaviour (e.g. [2]). Such investigations can provide accurate estimates provided the perturbation is sufficiently small, but suffer from numerical difficulties, since the sensitivity coefficient is derived as a ratio of small numbers (leading to errors introduced by limits in precision). The derivation presented here does not suffer from this numerical handicap.


## 4 Sensitivity Invariants

When systems are subjected to sensitivity analysis, their form often imposes certain restrictions, known as *sensitivity invariants*, on the resulting sensitivity functions [17]. Within the MCA community, the standard description of sensitivity invariants of biochemical systems are the Summation and Connectivity Theorems. In the classical case of steady-state analysis, these Theorems describe algebraic constraints on the sensitivity coefficients [15]. An equivalent statement of these results can be given as a description of the response of the system to perturbations in parameters of a particular form. In the current context, generalizing these results leads to the following statements regarding the asymptotic response coefficients.

**Summation Theorem:** If the parameter $p$ is chosen so that $\frac{\partial \mathbf{v}(t)}{\partial p}$ lies in the nullspace of $\mathbf{N}$ for each time $t$ during the oscillation $\mathbf{s}_{\mathrm{per}}(t, p)$, then

$$\begin{aligned} \mathbf{R^{s*}}(t) &= \mathbf{0} \\ \mathbf{R^{v*}}(t) &= \frac{\partial \mathbf{v}(t)}{\partial p}, \end{aligned}$$

for each $t \in [0, T(p))$. Moreover, $\frac{dT}{dp} = 0$.

**Connectivity Theorem:** If the parameter $p$ is chosen so that there is some $n$-vector $\mathbf{m}$ for which
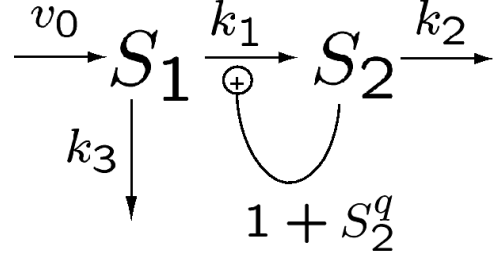


**Figure 1:** Abstracted glycolytic chain

$\frac{\partial \mathbf{v}(t)}{\partial p} = \frac{\partial \mathbf{v}(t)}{\partial \mathbf{s}} \mathbf{m}$ for each time $t$ during the oscillation $\mathbf{s}_{\mathrm{per}}(t, p)$, then

$$\begin{aligned} \mathbf{R^{s*}}(t) &= (\mathbf{H}_{\mathrm{per}}(t) - \mathbf{I})\mathbf{m} \\ \mathbf{R^{v*}}(t) &= \frac{\partial \mathbf{v}(t)}{\partial \mathbf{s}} \mathbf{H}_{\mathrm{per}}(t)\mathbf{m}, \end{aligned}$$

for each $t \in [0, T(p))$. Moreover, $\frac{dT}{dp} = 0$.

Note that in these special cases the sensitivity at arbitrary times $t$ is derived, not just at the extremal points. The proofs are omitted due to lack of space.


## 5 Application: a simple model of glycolytic oscillations

The simple two-species model shown in Figure 1 has been developed as an abstraction of the glycolytic pathway [9]. The kinetics are described by mass-action, with the activation by $S_2$ appearing multiplicatively (i.e. the rate of production of $S_2$ is given by $k_1 S_1 (1 + S_2^q)$). Provided the parameters lie in an appropriate range, the system exhibits limit cycle behaviour, as shown in [9].

With nominal parameter values of $v_0 = 8$, $k_1 = 1$, $k_2 = 5$, $k_3 = 1$, and $q = 3$, we consider the effect of perturbations in $k_3$, which is a measure of the external consumption of $S_1$. Simulations lead to the behaviour shown in Figure 2, where we see the oscillatory behavior of $S_1$, its sensitivity function $\mathbf{R}^{s_1}$, and the corresponding variation $\mathbf{\Delta R}^{s_1}$. Further analysis provides the sensitivity values in the table below. The only flux sensitivity which is reported is that of the reaction producing $S_2$ (denoted $v_3$) – the other nonconstant reaction rates are linear functions of the species concentrations.
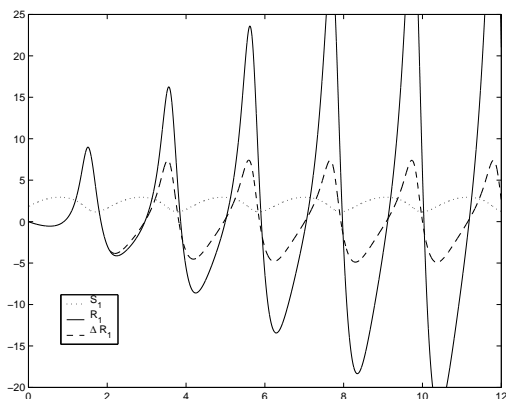
**Figure 2:** $S_1$, the sensitivity function $\mathbf{R}^{s_1}$, and its variation $\mathbf{\Delta R}^{s_1}$

| w.r.t. $k_3$ | sensitivity | relative sensitivity |
|---|---|---|
| $S_1$ max | -1.3 | -0.44 |
| $S_1$ min | 5.7 | 4.8 |
| $S_2$ max | -4.4 | -2.4 |
| $S_2$ min | 0.61 | 0.83 |
| $v_3$ max | -33 | -3.3 |
| $v_3$ min | 4.7 | 1.3 |

The sensitivity of the period with respect to $k_3$ was found to be: $-1.2$ (relative sensitivity $-0.56$). A complete analysis would determine the sensitivities with respect to the remaining parameters in the model.

## References

[1]   Acerenza, L., Sauro, H. M. and Kacser, H., "Control analysis of time-dependent metabolic systems," *J. Theor. Biol.*, **151** (1989), pp. 423–444.

[2]   Bier, M., Teusink, B., Kholodenko, B. N. and Westerhoff, H. V., "Control analysis of glycolytic oscillations," *Biophys. Chem.*, **62** (1996), pp. 15–24.

[3]   Buré, É. G. and Rozenvasser, E. N., "The study of the sensitivity of oscillatory systems," *Automat. Remote Contr.*, **7** (1974), pp. 1045–1052.

[4]   Demin, O. V., Westerhoff, H. V. and Kholodenko, B. N., "Control analysis of stationary fixed oscillations," *J. Phys. Chem. B*, **103** (1999), pp. 10695–10710.

[5]   Edelson, D. and Thomas, V. M., "Sensitivity analysis of Oscillating Reactions. 1. The period of the Oregonator," *J. Phys. Chem.*, **85** (1981), pp. 1555-1558.

[6]   Fell, D. A., *Understanding the Control of Metabolism*, Portland Press, London, 1997.

[7]   Goldbeter, A., *Biochemical Oscillations and Cellular Rhythms*, Cambridge University Press, Cambridge, 1996.

[8]   Hartman, P., *Ordinary Differential Equations*, John Wiley & Sons, New York, 1973.

[9]   Heinrich, R., Rapoport, S. M. and Rapoport, T. A., "Metabolic regulation and mathematical models," *Prog. Biophys. & Mol. Biol.*, **32** (1977), pp. 1–82.

[10]   Heinrich, R. and Reder, C., "Metabolic control analysis of relaxation processes," *J. Theor. Biol.*, **151** (1991), pp. 343–350.

[11]   Heinrich, R. and Schuster, S., *The Regulation of Cellular Systems*, Chapman & Hall, New York, 1996.

[12]   Ingalls, B. P. and Sauro, H. M., "Sensitivity analysis of stoichiometric systems: an extension of metabolic control analysis to non-steady state trajectories," *J. Theor. Biol.*, **222** (2003), pp. 23–36.

[13]   Kholodenko, B. N., Demin, O. V. and Westerhoff, H. V., "Control analysis of periodic phenomena in biological systems," *J. Phys. Chem. B*, **101** (1997), pp. 2070–2081.

[14]   Kramer, M. A., Rabitz, H. and Calo, J. M., "Sensitivity analysis of oscillatory systems," *Appl. Math. Modelling*, **8** (1984), pp. 328–340.

[15]   Reder, C., "Metabolic control theory: a structural approach," *J. Theor. Biol.*, **135** (1988), pp. 175–201.

[16]   Reijenga, K. A., Westerhoff, H. V., Kholodenko, B. N. and Snoep, J. L., "Control analysis for autonomously oscillating biochemical networks," *Biophys. J.*, **82** (2002), pp. 99–108.

[17]   Rosenwasser, E. and Yusupov, R., *Sensitivity of Automatic Control Systems*, CRC Press, Boca Raton, Florida, 2000.

[18]   Savageau, M. A., *Biochemical Systems Analysis. A Study of Function and Design in Molecular Biology*, Addison-Wesley, Reading, MA, 1976.