Journal of Mathematical Biology manuscript No.
(will be inserted by the editor)

A general model for the dynamics of cell volume, global stability, and optimal control

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Received: date / Accepted: date

Abstract Cell volume and concentration regulation in the presence of changing extracellular environments has been studied for centuries, and recently a general nondimensional model was introduced that encompassed solute and solvent transmembrane flux for a wide variety of solutes and flux mechanisms. Moreover, in many biological applications it is of considerable interest to understand optimal controls for both volume and solute concentrations. Here we examine a natural extension of this general model to an arbitrary number of solutes or solute pathways, show that this system is globally asymptotically stable and controllable, define necessary conditions for time-optimal controls in the arbitrary-solute case, and using a theorem of Boltyanski prove sufficient conditions for these controls in the commonly encountered two-solute case.

Keywords Cellular mass transport · optimization · stability · cryobiology · sufficiency theorem

1 Introduction

Recently, a general model of cell volume regulation was introduced that accounts for active and passive transport of water and a solute across the cell membrane

This work appeared as part of a doctoral dissertation [Benson(2009)]

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[Hernández et al(2007)Hernández, Roca, Gil, Vázquez, and Martínez,Hernandez(2003)]

$$\begin{aligned} x' &= \alpha - \beta x/y, \\ y' &= -\gamma + \sigma x/y + \varepsilon/y, \end{aligned} \tag{1}$$

where y is a (positive) non-dimensional water volume variable, x is a non-negative non-dimensional solute mass variable, α and γ are extracellular concentration variables, and β, σ and ε are cell dependent rate parameters. As discussed by [Hernández(2007)], this model is a general form for many existing in the literature [Katkov(2000),Katkov(2002), Kleinhans(1998)]. It is simple to extend this model to multiple permeating solute species and pathways by defining $w_1 = y$ to be the positive non-dimensional water volume of the cell, and w_i, $i = 2,\ldots,n$ to be the $n-1$ non-negative solute species or pathways, and x_{np} to be the non-negative non-permeating solute species analogous to ε [Katkov(2000)]. In this case, we define $M_i : [0,\infty) \to [0,\infty)$, $i = 2,\ldots,n$, to be extracellular concentration variables analogous to α from system (1), and let the sum of the extracellular concentrations $\sum_{i=1}^{n} M_i$ be the analog of γ from system (1), where $M_1 : [0,\infty) \to [0,\infty)$ is the concentration of nonpermeating solute. Finally, define $b_i > 0$, $i = 2,\ldots,n$, to be the rate constants analogous to β and σ. Using the temporal parmeter s and restricitng the state variables $\{w_1, w_2, w_3, \ldots, w_n\}$ to the positive orthant, we have the general multispecies model

$$\begin{aligned} w_1' &= \frac{x_{\text{np}}}{w_1} + \sum_{j=2}^{k} \frac{w_j}{w_1} - \sum_{i=1}^{n} M_i, \\ w_2' &= b_2 \left(M_2 - \frac{w_2}{w_1} \right), \\ &\vdots \\ w_n' &= b_n \left(M_n - \frac{w_n}{w_1} \right), \end{aligned} \tag{2}$$

which we also express in the more compact form

$$w' = h(w, M).$$

Applications of our multispecies model can be applied to cryobiology in particular [Katkov(2000)] but since there are a large number of intracellular and extracellular chemical species that permeate across the cell boundary, it is natural to assume that if cells are placed in any non-physiologic environment, there will be transmembrane transport of water and more than one solute.

In this manuscript, we will investigate the dynamics of these physiologically relevant models. [Hernández(2007)] showed that model (1) is locally stable at its rest point provided the rest point resides in the physically relevant region ($x > 0$ and $y > 0$). One would expect that this stability is in fact global asymptotic stability, and that a similar result is true for the model (2). We are able to prove both results.

Additionally, it is often desirable to determine optimal protocols for the control of intracellular concentrations of permeating reagents in cells governed by model (1)) or (2). Examples can range from pharmicokinetics [Ledzewicz and Schättler(2007)] to cryobiology [Levin(1982)]. We give the conditions for a controllability result, show existence of an optimal control, and synthesize an optimal control in the commonly encountered case of one permeating and one non-permeating solute.

Our analysis hinges on the observation, which we applied previously [Benson et al(2005)Benson, Chicone, and Critser], that our nonlinear system (2) can be made linear by multiplying the right-hand side by w_1. Specifically, we may factor $h(w,M)$ into $g(w)f(w,M)$ where $g(w) = 1/w_1$, yielding a system of the form $w' = g(w)f(w)$, where $g : \mathbb{R}^n \to \mathbb{R}$ is a positive scalar valued function. Because g is strictly positive, the qualitative behavior of the system $x' = f(x)$ is the same as system (2). To be precise, suppose that $v \in \mathbb{R}^n$ and $s \mapsto \eta(s)$ is the solution of the initial value problem

$$w' = h(w) := g(w)f(w), \qquad w(0) = v \tag{3}$$

where $g : R^n \to R^+$ and $f : R^n \to R^n$ are smooth, and $t \mapsto \phi(t)$ is the solution of the initial value problem

$$x' = f(x), \qquad x(0) = v.$$

Using the time transform

$$q(t) := \int_0^t \frac{1}{g(\phi(\tau))}\, d\tau, \tag{4}$$

we have a basic fact: $\phi(t) = \eta(q(t))$. To prove it, we use the positivity of g to conclude that q is invertible with inverse ρ (see [Chicone(1999)]) and define $\gamma(s) = \phi(\rho(s))$. This function γ is such that $\gamma(0) = v$ and

$$\gamma'(s) = \rho'(s)\phi'(\rho(s)) = \frac{1}{q'(\rho(s))} f(\phi(\rho(s))) = g(\gamma(s))f(\gamma(s)).$$

Thus, γ is the solution of the initial value problem (3). In other words, $\eta(s) = \phi(\rho(s))$ and $\phi(t) = \eta(q(t))$ as required; or, in less formal language, $x(t) = w(q(t))$. In the context of system (2), the function $t \mapsto x(t)$ solves the linear system

$$\begin{aligned}
\dot{x}_1 &= x_{\text{np}} + \sum_{j=2}^{n} x_j - \sum_{i=1}^{n} M_i x_1, \\
\dot{x}_2 &= b_2 (M_2 x_1 - x_2), \\
&\vdots \\
\dot{x}_n &= b_n (M_n x_1 - x_n),
\end{aligned}$$

which we also write in the vector form,

$$\dot{x} = f(x,M) := A(M)x + x_{np}e_1, \tag{5}$$

where $\dot{x} = \frac{dx}{dt}$, $M := (M_1, M_2, \ldots, M_n)$, $A(M)$ is the matrix

$$A(M) = \begin{pmatrix} -\sum_{i=1}^{n} M_i & 1 & 1 & \ldots & 1 \\ b_2 M_2 & -b_2 & 0 & \ldots & 0 \\ b_3 M_3 & 0 & -b_3 & \ldots & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ b_n M_n & 0 & 0 & \ldots & -b_n \end{pmatrix}.$$

and e_1 is the usual first unit-basis vector.

2 Dynamics for $M(t) \equiv M$

2.1 Stability

As mentioned in the introduction, [Hernández(2007)] proved local stability for model 2 in case $n = 2$ and the corresponding M_i are constant functions by showing that the spectrum of the linearized equations at the steady state lies in the (open) left-half of the complex plane. Because we have reparametrized to obtain a linear system that has the same qualitative dynamics as the original system, once we show that this spectrum lies in the left half-plane for our linear system, we will have a stronger result: the rest point is globally asymptotically stable. In fact, we will prove a result on global asymptotic stability for the general case where $n \geq 2$, and the M_i are constant functions.

The zeros of system 5 must satisfy $x_1 = x_{\text{np}}/M_1$, and $x_j = M_j x_1$ for $j = 2, \ldots, n$; or, in other words, the rest point is $x^* = (x_{\text{np}}/M_1)(1, M_2, \ldots, M_n)^T$. Because we have a linear system, our first theorem has a simple proof.

Theorem 1 *If the function M is constant and $M_1 > 0$, then the rest point*

$$x^* = (x_{np}/M_1)(1, M_2, \ldots, M_n)^T$$

of the system $\dot{x} = A(M)x + x_{np}e_1$ is globally asymptotically stable. Moreover, the rest point x_* of system (2) *is globally asymptotically stable.*

Proof Let D be the diagonal $n \times n$-matrix with diagonal $(1, (b_2 M_2)^{-1/2}, \ldots, (b_n M_n)^{-1/2})$ and note that

$$DA(M)D^{-1} = \begin{pmatrix} -\sum_i M_i & \sqrt{b_2 M_2} & \sqrt{b_3 M_3} & \ldots & \sqrt{b_n M_n} \\ \sqrt{b_2 M_2} & -b_2 & 0 & \ldots & 0 \\ \sqrt{b_3 M_3} & 0 & -b_3 & \ldots & 0 \\ \vdots & \vdots & \vdots & \ddots & \vdots \\ \sqrt{b_n M_n} & 0 & \ldots & 0 & -b_n \end{pmatrix} \tag{6}$$

is a symmetric negative definite matrix.

3 Optimal Control

3.1 Controllability

The notion of controllability and stability go hand in hand. It is simple to check that a globally asymptotically stable system is controllable by considering the rank of the controllability matrix function $G : \mathbb{M}^{n \times n} \times \mathbb{M}^{n \times m} \to \mathbb{M}^{n \times mn}$ given by

$$G(A, B) := [B|AB|A^2B| \ldots |A^{n-1}B].$$

In our case, we adopt Mohler's notion of a bilinear control system [Mohler(1973)]: a system is bilinear in state x and control vector $u \in \mathbb{R}^m$ if

$$\begin{aligned}\dot{x} &= f(x,u) \\ &= Ax + \sum_j B_j u_j x + Cu\end{aligned} \tag{7}$$

for appropriately sized matrices A, B_j and C. In this bilinear case, we may apply a result from Mohler. Let $\mathscr{A} \subset \mathbb{R}^m$ denote the admissible control parameter set and define $x(t;x_0,u)$ to be the solution of system (7) with control u and initial condition x_0, and

$$\mathscr{C}_{x^*} := \{y \in \mathbb{R}^n : y = x(0),\, x(t;x(0),u) = x^*,\, t < \infty,\, u(t) \in \mathscr{A}\}.$$

Then we have the following proposition

Proposition 1 (Mohler) *Suppose that f is bilinear as defined in 7, $u(t) \in \mathscr{A}$, and $x^* \in \mathbb{R}^n$ and $u^* \in$ interior Ω are such that $f(x^*,u^*) = 0$. Define $\bar{A}_f = (\partial f/\partial x)(x^*,u^*)$ and $\bar{B}_f = (\partial f/\partial u)(x^*,u^*)$. If $\operatorname{rank} G(\bar{A}_f, \bar{B}_f) = n$ and x^* is an asymptotically stable rest point of the system $\dot{x} = f(x,u^*)$, then $\mathscr{C}_{x^*} = \mathbb{R}^n$.*

We apply this proposition to the bilinear system (5) noting that $A_f(x^*,u^*) = A(M^*)$ and

$$B_f(x^*,u^*) = \begin{pmatrix} -1 & -1 & -1 & -1 \\ 0 & b_2 & 0 & 0 \\ \vdots & 0 & \ddots & 0 \\ 0 & \dots & 0 & b_n \end{pmatrix}.$$

Since $x_1^* B$ is an $n \times n$ upper triangular matrix with non-zero diagonal entries, the first n columns are linearly independent and thus the rank of $G(A(M^*), x_1^* B_1) = n$.

Finally, we combine Theorem 1 with Proposition 1 to obtain the following result.

Theorem 2 *For system (5), $\mathscr{C}_{x^*} = \mathbb{R}^n$.*

3.2 Existence of an optimal control

The existence of an optimal control for systems of the form 7 with bounded controls is a standard result (see [Lee and Markus(1968), Corollary 2, p. 262]).

3.3 A control problem

We prescribe an m-tuple of numbers $\bar{M}_i > 0$ for $i = 1,2,\dots,n$, the admissible control parameter set

$$\mathrm{CP} = \{M = (M_1, M_2, \dots, M_n) \in \mathbb{R}^n : 0 \le M_i \le \bar{M}_i \text{ for } i = 1,2,\dots,n\}, \tag{8}$$

and the state space $S \subset (0,\infty)\times[0,\infty)^{n-1}$ (i.e. we do not allow $x_1 = 0$). In addition, we define $x(t) = x(t;x_0,M)$ to be the solution of the initial value problem (7) and

$$\mathscr{C}_y(t) = \{x_0 \in S : x(t) := x(t;x_0,u) = y\},$$

the set of initial conditions that can be steered to $y \in S$ at time t via a measurable, admissible control function $M : \mathbb{R}^+ \to \mathrm{CP}$.

We will investigate the time-optimal control problem of steering an initial state x^i to a final state x^f in minimal "real" time using controls in the admissible set $\mathbb{A}$, the set of measurable functions $M : \mathbb{R} \to \mathrm{CP}$. This control problem has wide applications in biology because it is often desirable to implement the control of an extracellular environment in such a way as to minimize exposure time. This problem is encountered in ex-vivo settings including cell culture and processing, where extracellular envirionments must be controlled to achieve final intracellular concentrations of various reagents. For example, in cryobiology one wishes to equilibrate cell suspensions with multimolal concentrations of cryoprotective agents such as glycerol or dimethyl sulfoxide while minimizing exposure times correlating to time-dependent toxicity effects.

Therefore we define the original time-optimal control problem

Problem 1 Given an initial state w^i in the state space S and final state $w^f \in S$, the set of admissible controls $\mathbb{A}$ and defining $s^* \in \mathbb{R}$ to be the first time that $w(s^*) = w^f$ for the solution of the previously defined initial value problem defined in system (2), determine a control that minimizes s^* over $M \in \mathbb{A}$.

Using the time-transform function q in (4), we have the equivalent problem

Problem 2 Given an initial state x^i in the state space S and final state $x^f \in S$, the set of admissible controls $\mathbb{A}$ and defining $t^* \in \mathbb{R}$ to be the first time that $x(t^*) = x^f$ for the solution of the previously defined initial value problem

$$\dot{x} = f(x,M) = A(M)x + x_{np}e_1, \quad x(0) = x^i, \tag{9}$$

determine a control that maximizes the functional

$$P(M) := -s^* = -q(t^*) = -\int_0^{t^*} x_1(t)\,dt \tag{10}$$

over $\mathbb{A}$.

By construction, these problems are, in fact, the same and thus if $M(t)$ is the maximizer in problem (2), then $M(q^{-1}(t))$ is the minimizer of system (1). We state this in the following lemma.

Lemma 1 *Problem 1 and Problem 2 are equivalent.*

Proof Suppose M_x^* minimizes $P(M) = q(t^*)$ (defined in eq. (10) for system (9)) and define $s^* = q(t^*)$. Suppose M_w^* minimizes s for system (2) in Problem 1 with transit time $\bar{s}$. Then $M_w^* \circ q^{-1}$ is an admissible control for Problem 2, with cost $P_x(M_w^* \circ q^{-1}) = \bar{s} = q(\bar{t})$, and $q(t^*) \leq q(\bar{t})$. Alternatively, $M_x^* \circ q$ is an admissible control for problem (1) with cost $P_w(M_x^* \circ q) = q(t^*) = s^*$ where $s^* \geq \bar{s}$. Thus $q(t^*) \geq q(\bar{t})$, and thus $s^* = \bar{s}$.

Our basic tool for attacking this time-optimal control problem is the Pontryagin Maximum Principle (see Lee and Markus for example [Lee and Markus(1968)]). For our model system (9), we wish to minimize the payoff functional (10) which is the negative "real" time measured from time $s = 0$ given by our conversion formula (4).

For our control system (9), we define the (control theory) Hamiltonian

$$H(x,p,M) = f(x,M)\cdot p - x_1.$$

The state of the system satisfies the differential equation $\dot{x} = \nabla_p H(x,p,M) = f(x,M)$ and the costate p satisfies $\dot{p} = -\nabla_x H(x,p,M)$. In our case, the state equation has the explicit form $\dot{x} = A(M)x + x_{np}e_1$ and the costate equation is given by $\dot{p} = -A(M)^T p + e_1$.

For Problem 2, we can immediately deduce the nature of the optimal control by applying the maximum principle: we have the Hamiltonian

$$H(x,p,M) = (A(M)x + x_{np}e_1)\cdot p - x_1 = (x_1 B_1 M + B_2 x + x_{np}e_1)\cdot p - x_1, \qquad (11)$$

and we must find $M \in \mathbb{A}$ such that $H(x,p,M)$ is maximized. Thus we maximize

$$\begin{aligned} H(x,p,M) &= (A(M)x + x_{np}e_1)\cdot p - x_1 \\ &= \left(-\sum_{i=1}^{n} M_i x_1 + \sum_{i=2}^{n} x_i + x_{np}\right) p_1 + \sum_{i=2}^{n} (M_i b_i x_1 - b_i x_2) p_i - x_1 \\ &= -\sum_{i=1}^{n} M_i x_1 p_1 + \ldots + \sum_{i=2}^{n} (M_i b_i x_1 - b_i x_i) p_i + \ldots \\ &= -M_1 x_1 p_1 + x_1 \sum_{i=2}^{n} M_i (b_i p_i - p_1) + \ldots, \end{aligned}$$

where the ellipses represent terms that we may ignore because they are not affected by the controls M_i. This expression is maximized when

$$M_1(t) = \begin{cases} 0, & p_1 > 0 \\ \bar{M}_1, & p_1 \le 0 \end{cases} \quad \text{and} \quad M_i(t) = \begin{cases} 0, & b_i p_i - p_1 < 0 \\ \bar{M}_i, & b_i p_i - p_1 \ge 0. \end{cases} \qquad (12)$$

3.4 Synthesis of the optimal control in the case $n = 2$.

Synthesizing the optimal control for $n > 2$ becomes a technical challenge due to the number of state and costate cases one must consider. Therefore, we will construct the optimal control in the commonly encountered and biologically important case where there is one permeating and one non-permeating solute and $n = 2$. Our approach is a classical geometrical method developed by Boltyanskii [Boltyanskii(1966)], (see [Vakhrameev(1995)] for review).

In the unconstrained case, the maximum principle limits the synthesis to four possible control schemes, $M^I, \ldots, M^{IV}$ associated with four regions ($\Pi^I, \ldots, \Pi^{IV}$) in costate space:

$$\Pi_I := \{p \in \mathbb{R}^2 : p_1 < 0, b_2 p_2 - p_1 > 0\},$$
$$\Pi_{II} := \{p \in \mathbb{R}^2 : p_1 > 0, b_2 p_2 - p_1 > 0\},$$
$$\Pi_{III} := \{p \in \mathbb{R}^2 : p_1 > 0, b_2 p_2 - p_1 < 0\},$$
$$\Pi_{IV} := \{p \in \mathbb{R}^2 : p_1 < 0, b_2 p_2 - p_1 < 0\},$$

(see Fig. 1), and the control schemes for initial points in each region (Table 1).

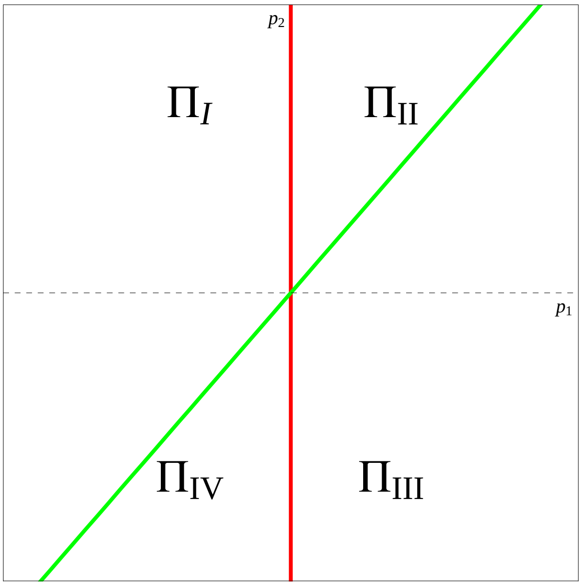

Fig. 1: Plot of the costate regions $\Pi_I, \Pi_{II}, \Pi_{III}, \Pi_{IV}$, defined by the maximum principle.

Define $S^* := \{x \in S : x_1 > x_{np}/\bar{M}_1, 0 \le x_2 < \bar{M}_2 x_1\}$ to be the region in the state space where x^i and x^f may reside, and define sets $P^0 = \{x^f\}$, $P^1 = \cup_{i=1}^4 \sigma^i$, and $P^2 = S$, where

$$\sigma^i := \{x \in S : \phi_t^\lambda(x_f) = x \text{ for } t < 0\},$$

and $\phi_t^\lambda(x_f)$ is the solution of $\dot{x} = f(x, \lambda)$ from initial point x^f under control scheme $\lambda = M^I, M^{II}, M^{III}$, or M^{IV} (see Fig. 2). We define regions $\mathscr{A}, \mathscr{B}, \mathscr{C}$ and $\mathscr{D}$ as follows. Let $\mathscr{A} \subset S$ be the region bounded by $\partial S \cup \sigma^I \cup \sigma^{II} \cup \{x^f\}$ that does not contain $\sigma^{III} \cup \sigma^{IV}$. Let $\mathscr{B} \subset S$ be the region bounded by $\partial S \cup \sigma^{II} \cup \sigma^{III} \cup \{x^f\}$ that does not contain $\sigma^{IV} \cup \sigma^I$. Let $\mathscr{C} \subset S$ be the region bounded by $\partial S \cup \sigma^{III} \cup \sigma^{IV} \cup \{x^f\}$ that does not contain $\sigma^I \cup \sigma^{II}$. Let $\mathscr{D} \subset S$ be the region bounded by $\partial S \cup \sigma^{IV} \cup \sigma^I \cup \{x^f\}$ that does not contain $\sigma^{II} \cup \sigma^{III}$.

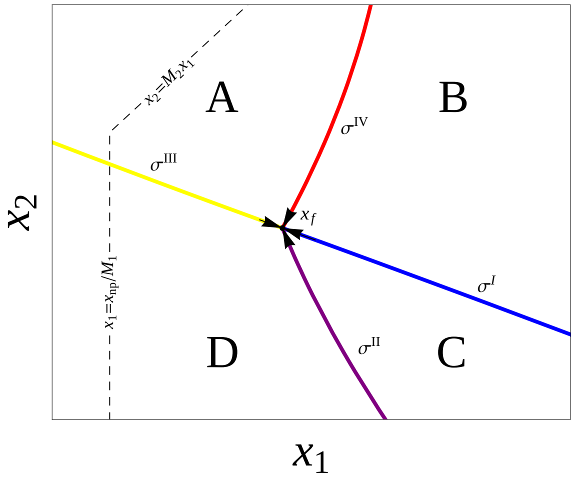

Fig. 2: Typical plot of the state regions. The geometry of the regions changes as a function of x^f, the "source" of the σ^i, though the regions remain bounded by the same σ^i. Also, S^* is bounded on the left and above by the dashed lines $x_1 = x_{np}/M_1$ and $x_2 = M_2 x_1$.

Table 1: The Pontryagin Maximum Principle along with the Hamiltonian for the $n = 2$ system define the possible optimal control schemes M^I, M^{II}, M^{III}, and M^{IV}.

Control Scheme	M_1	M_2
M^I	$\bar{M}_1$	$\bar{M}_2$
M^{II}	0	$\bar{M}_2$
M^{III}	0	0
M^{IV}	$\bar{M}_1$	0

Using the notation just developed, we define $v : S \to U$:

$$v(x) = \begin{cases} M^I, & x \in \sigma^I \\ M^{II}, & x \in \mathscr{C} \cup \mathscr{D} \cup \sigma^{II} \\ M^{III}, & x \in \sigma^{III} \\ M^{IV}, & x \in \mathscr{A} \cup \mathscr{B} \cup \sigma^{IV} \end{cases}, \tag{13}$$

which defines control schemes (see Table 1) for initial points $x^i \in \text{int}\, S^*$ in the subregions (see Table 2).

Table 2: Control schemes in the subregions of S. Our synthesis will be constructed for initial and final points x^i and x^f in S divided into four regions $\mathscr{A},\mathscr{B},\mathscr{C}$ and $\mathscr{D}$. For each region the First Control is used until a defined switching time τ after which the Second Control is used. In the unconstrained case, the control $M(t)$ is piecewise constant.

Region	First Control	Second Control
$\mathscr{A}$	M^{IV}	M^{III}
$\mathscr{B}$	M^{IV}	M^{I}
$\mathscr{C}$	M^{II}	M^{I}
$\mathscr{D}$	M^{II}	M^{III}

Theorem 3 *The trajectory defined by $\dot{x} = f(x, v(x))$ where v is defined by display* (13) *is optimal.*

To prove this theorem below, we will use the classic result of Boltyanskii, which states that any "regular" and "distinguished" control is optimal [Boltyanskii(1966)]. For the convenience of the reader, the result of Boltyanskii is provided in the Appendix.

We next introduce two unexpected results. For initial points in regions $\mathscr{A}$ and $\mathscr{D}$ there are simple formulas that describe the total transit time t^* that are not dependent on the maximal concentration; i.e. provided the starting point lies in either $\mathscr{A}$ or $\mathscr{D}$ the total transit time is solely determined by x^i, x^f, x_{np}, and b_2!

Theorem 4 *For $x^i \in \mathscr{D}$, the total optimal transit time under the associated control scheme is $t^* = \frac{x_1^f - x_1^i}{x_{np}} + \frac{x_2^f - x_2^i}{b_2 x_{np}}$. For $x^i \in \mathscr{A}$, the total optimal transit time under the associated control scheme is $t^* = \frac{1}{b_2}\ln(x_2^i/x_2^f)$.*

There may be similar formulae for other regions, but the equations become much more complicated in these cases.

This first lemma, which will be used to prove Theorem 3 shows that there are no rest points of the controlled system in the interior of S.

Lemma 2 *For $x^f \in S^*$, there are no rest points of system $\dot{x} = f(x, v(x))$ in* $\operatorname{int} S \setminus \sigma^I$.

Proof In the union $\mathscr{C} \cup \mathscr{D} \cup \sigma^{II} \cup \sigma^{III}$, $M_1 = 0$, and there is no rest point. In the union $\mathscr{A} \cup \mathscr{B} \cup \sigma^{IV}$, $M_2 = 0$, and the rest point is at $(x_{\text{np}}/M_1)(1,0)^T$. Region $\mathscr{A}$ is bounded by σ^{III} and σ^{IV}, for which $M_2 = 0$, because of this, in negative time, $\dot{x}_2 > 0$, and thus for all $x^f \in S^*$, $\mathscr{A}$ is bounded away from the x_1-axis. For region $\mathscr{B}$, because σ^{IV} is bounded away from the x_1-axis, it remains to show that if σ^I intersects the x_1-axis, it does so for $x_1 > x_{\text{np}}/\bar{M}_1$ (e.g. $\mathscr{B}$ is bounded away from the associated rest point). But, for $x^f \in S^*$, $x_1 > x_{np}/\bar{M}_1$ and $x_2 < \bar{M}_2 x_1$. In this case, in negative time

$\dot{x}_1 = (M_1 + M_2)x_1 - x_2 - x_{np} > x_{np} + M_2x_1 - M_2x_1 - x_{np} = 0$, thus σ^I intersects the x_1-axis at some $x_1 > x_{np}/\bar{M}_1$.

The next lemma states that at every point in the interior of S the vector fields defined by the four controls are not parallel.

Lemma 3 *If $x \in \sigma^{III} \cap \operatorname{int} S$, then $f(x, M^{IV})$ and $f(x, M^{III})$ are not parallel.*
If $x \in \sigma^{I} \cap \operatorname{int} S$, then $f(x, M^{IV})$ and $f(x, M^{I})$ are not parallel.
If $x \in \sigma^{III} \cap \operatorname{int} S$, then $f(x, M^{II})$ and $f(x, M^{III})$ are not parallel.
If $x \in \sigma^{I} \cap \operatorname{int} S$, then $f(x, M^{II})$ and $f(x, M^{I})$ are not parallel.

Proof Let $\eta \in \mathbb{R}$. If $x \in \partial S^*$ and $x_1 = x_{np}/M_1$, then $-f(x, M^I) \cdot (1,0) > 0$. Also if $x \in \partial S^*$ and $x_2 = M_2x_1$, then $-f(x, M^I) \cdot (0,1) < 0$. Thus, for all $x^f \notin \partial S^*$, $\sigma^I \cap (\partial S^* \setminus \mathbb{R} \times \{0\}) = \emptyset$. Now suppose $x \in \mathbb{R} \times \{0\}$. Then $-f(x, M^{III}) \cdot (0,1) > 0$, therefore for all $x^f \notin \mathbb{R} \times \{0\}$, $\sigma^{III} \cap \mathbb{R} \times \{0\} = \emptyset$. The solution of $f(x, M^{IV}) = \eta f(x, M^{III})$ is $x_1 = (x_{np}/M_1)(1-\eta)$, $x_2 = 0$. The solution of $f(x, M^{IV}) = \eta f(x, I)$ is $x_1 = x_{np}/M_1$, $x_2 = (M_2x_{np}\eta)/(M_1(\eta - 1))$. This solution is not in $\operatorname{int} S$ for all η. There is no solution of $f(x, M^{II}) = \eta f(x, M^{III})$. The solution of $f(x, M^{II}) = \eta f(x, M^I)$ is $x_1 = (x_{np}/\eta M_1)(1-\eta)$, $x_2 = (M_2x_{np}/M_1\eta)(\eta - 1)$. Factoring out $(x_{np}/\eta M_1)(1-\eta)$, we get $(x_{np}/\eta M_1)(1-\eta)(1, M_2)$. This parametrizes the boundary $x_2 = x_1M_2$ of S^f, and thus is not in $\operatorname{int} S$ for all η.

The next lemma states that given initial points in each region $\mathscr{A}, \mathscr{B}, \mathscr{C}, \mathscr{D}$, the flow along the solution given by the associated control intersects the expected boundary curve σ^i in finite time.

Lemma 4 *(1) Given an initial point $x \in \mathscr{A}$, there exists a time $\tau_1 > 0$ such that $\phi^{IV}_{\tau_1}(x)$ intersects σ^{III}. (2) Given an initial point $x \in \mathscr{B}$, there exists a time $\tau_1 > 0$ such that $\phi^{IV}_{\tau_1}(x)$ intersects σ^I. (3) Given an initial point $x \in \mathscr{C}$, there exists a time $\tau_1 > 0$ such that $\phi^{II}_{\tau_1}(x)$ intersects σ^I. (4) Given an initial point $x \in \mathscr{D}$, there exists a time $\tau_1 > 0$ such that $\phi^{II}_{\tau_1}(x)$ intersects σ^{III}.*

Proof By Lemma 2 there are no rest points within any of the regions under the control v. We claim that S is invariant for all controls. In fact, on the x_1-axis, all controls have $\dot{x}_2 \geq 0$, and on the x_2-axis, all controls have $\dot{x}_1 \geq 0$. From Lemma 2 and Theorem 1, regions $\mathscr{A}$ and $\mathscr{B}$ are bounded away from the asymptotically stable rest point associated with their control, and thus from any initial point in $\mathscr{A}$ or $\mathscr{B}$, the flow must cross $\partial\mathscr{A}$ or $\partial\mathscr{B}$ respectively. Because the control in both regions is M^{IV}, by the uniqueness of solutions of ODEs the flow will not cross σ^{IV}, and thus (1) and (2) are proved. Now note that for $x \in S^*$, $f(x, M^I)$ and $f(x, M^{II})$ have a positive second component, thus σ^I and σ^{II} (which flow in negative time) will always intersect the x_1-axis, and region $\mathscr{C}$ will be bounded away from $x_2 > x_2^f$. Moreover, for $x \in S$, the first component of $f(x, M^{III})$ is positive, σ^{III} must intersect the x_2 axis, and thus region $\mathscr{D}$ is bounded. Finally, for $x \in S^*$, $f(x, M^{II})$ has a positive second component. Thus for $x^i \in \mathscr{C} \cup \mathscr{D}$, $\phi_t^{M^{II}}(x^i)$, must intersect $\partial\mathscr{C} \cup \partial\mathscr{D}$, and as above, because the control in both regions is M^{II}, by the uniqueness of solutions of ODEs the flow will not cross σ^{II}, and thus (3) and (4) are proved.

Lemma 5 *The transit time $\tau(x^i)$ from x^i to x^f is a continuous function of the initial point x^i.*

Proof Let $y \in \sigma^i$, define $\tau_2(y)$ to be the time to reach x^f while flowing along σ^i, and define $F(t,x,y) := \phi(t,x,\lambda) - y$. By Lemma 4, for each $x^i \in G$ there exists a τ_1 such that $\phi(\tau_1,x,v(x)) \in \sigma^i$. Thus, we have $F(\tau_1,x,y) = 0$. Because there exist no rest points, $\frac{d}{dt}F(t,x,y)|_{t=\tau_1} \neq 0$ and we may apply the implicit function theorem yielding $\tau^1(x)$ is C^1 in a neighborhood of x, thus the transit time $\tau(x) = \tau_1(x) + \tau_2(\phi(\tau^1(x),x,II))$ is continuous.

Next we examine whether there exists a costate that will satisfy the Pontryagin Maximum Principle.

Lemma 6 *The lines ℓ_{II} defined by the solution of $H(x(0),p(0),M^{II}) = 0$ and ℓ_{IV} defined by the solution of $H(x(0),p(0),M^{IV}) = 0$ are not parallel to the line $b_2p_2 = p_1$.*

Proof Setting the initial Hamiltonian for control M^{II} equal to zero, we get

$$\begin{aligned} H(x(0),p(0),M^{II}) &= \begin{pmatrix} -M_2x_1^i + x_2^i + x_{np} \\ M_2b_2x_1^i - b_2x_2^i \end{pmatrix} \cdot \begin{pmatrix} p_1 \\ p_2 \end{pmatrix} + x_1^i, \\ &= (-M_2x_1^i + x_2^i + x_{np})p_1 + (M_2b_2x_1^i - b_2x_2^i)p_2 + x_1^i, \\ &= 0. \end{aligned}$$

We solve this for

$$b_2p_2 = \frac{M_2x_1^i - x_2^i - x_{np}}{M_2x_1^i - x_2^i}p_1 - \frac{x_1^i}{M_2x_1^i - x_2^i}.$$

Note that $\frac{M_2x_1^i - x_2^i - x_{np}}{M_2x_1^i - x_2^i} = 1$ if and only if $M_2x_1^i - x_2^i - x_{np} = M_2x_1^i - x_2^i$. But, reducing this equation we get $x_{np} = 0$ in contradiction since $x_{np} > 0$. For control M^{IV}, we have

$$\begin{aligned} H(x(0),p(0),M^{IV}) &= \begin{pmatrix} -M_1x_1^i + x_2^i + x_{np} \\ -b_2x_2^i \end{pmatrix} \cdot \begin{pmatrix} p_1 \\ p_2 \end{pmatrix} + x_1^i \\ &= (-M_1x_1^i + x_2^i + x_{np})p_1 - b_2x_2^ip_2 + x_1^i \\ &= 0. \end{aligned}$$

We solve this for $b_2p_2 = (-M_1x_1^i + x_2^i + x_{np})/x_2^i + x_1^i/x_2^i$. Now $(-M_1x_1^i + x_2^i + x_{np})/x_2^i = (x_{np} - M_1x_1^i)/x_2^i + 1 < 1$, since $x_{np} < M_1x_1^i$.

Proposition 2 *Given Problem (2) with an initial point $x^i \in S^* \setminus \cup_{i=I}^{IV}\sigma^i$, with its associated optimal control scheme λ, switching time τ^1, and the control scheme's associated initial and final costate regions (Π^i and Π^f, respectively), there exists a costate p such that for all $t > 0$, the Hamiltonian $H(x,p,\lambda) := f(x,\lambda) \cdot p + x_1 = 0$, and $p(t)$ solves $\dot{p} = -\nabla_x H(x,p,\lambda)$ such that $p(t) \in \Pi^i$ for $t < \tau^1$, $p(\tau^1) \in \partial\Pi^i$ and $p(t) \in \Pi^f$ for $t > \tau_1$.*

Proof We must show that for each control scheme and time $\tau^1 > 0$, there exists an initial costate such that $p(t) \in \Pi^i$ for $t < \tau^1$, $p(\tau^1) \in \partial\Pi^i$ and $p(t) \in \Pi^f$ for $t > \tau^1$. We need only consider $\Pi^i = \Pi_{II}$ and the $\Pi^i = \Pi_{IV}$ cases . In both cases $H(x(0), p(0), \lambda) = 0$ defines a line ℓ in the costate space with non-infinite slope that, by Lemma 6, also does not equal $1/b_2$. In the Π_{II} case, the costate dynamics are governed by

$$\begin{pmatrix} \dot{p}_1 \\ \dot{p}_2 \end{pmatrix} = \begin{pmatrix} M_2 p_1 - M_2 b_2 p_2 + 1 \\ -p_1 + b_2 p_2 \end{pmatrix}. \tag{14}$$

It is easy to check that this system has an invariant line defined by $p_2 = p_1/b_2 + 1/(b_2^2 + \bar{M}_2 b_2)$ that by Lemma 6 will intersect ℓ. We claim that for $p \in \Pi_{II}$ such that $p_2 > p_1/b_2 + 1/(b_2^2 + \bar{M}_2 b_2)$, there exists a $t < \infty$ such that the solution of (14) intersects the p_2-axis at time τ^1.

We first let $p_2 > p_1/b_2 + 1/M_2 b_2$. In this case $\dot{p}_1 < 0$ and $\dot{p}_2 > 0$, so we must flow toward the p_2-axis, and since $\lim_{p_2 \to \infty} \dot{p}_2/\dot{p}_1 = -1/M_2$, the flow does not “blow up” to $p_2 = \infty$ before crossing the p_2-axis. Now for $p_1/b_2 + 1/M_2 b_2 > p_2 > p_1/b_2 + 1/(M_2 b_2 + b_2^2)$, $\dot{p}_2 > 0$. Thus for such p, p_2 will increase until $p_2 > p_1/b_2 + 1/M_2 b_2$ and we are done. Next let $p_2 < p_1/b_2 + 1/(M_2 b_2 + b_2^2)$. Note that $\lim_{p_1 \to \infty} \dot{p}_2/\dot{p}_2 = -1/M_2$, and $\lim_{p_1 \to \infty} \dot{p}_2 = -\infty$, therefore as p_1 gets large, the direction of the flow is downward and will cross the line $p_2 = p_1/b_2$.

For the Π_{IV} case, the costate dynamics are governed by

$$\begin{pmatrix} \dot{p}_1 \\ \dot{p}_2 \end{pmatrix} = \begin{pmatrix} M_1 p_1 + 1 \\ -p_1 + b_2 p_2 \end{pmatrix}.$$

We can check that this system has an invariant line at $p_1 = -1/\bar{M}_1$. For p such that $p_1 > -1/\bar{M}_1$, $\dot{p}_1 > 0$, and for p such that $p_1 < -1/\bar{M}_1$, $\dot{p}_1 < 0$ and $\lim_{p_1 \to -\infty} \dot{p}_2 = +\infty$ with $\lim_{p_1 \to -\infty} \dot{p}_2/\dot{p}_1 = -1/M$, therefore as $-p_1$ gets large, the direction of the flow is upward and will cross the p_1-axis.

Thus, in both cases we can find an initial point along ℓ such that, following the respective dynamics, p reaches $\partial\Pi^i$ at $t = 0$ and at $t = \infty$. By continuity there exists an initial point such that p reaches $\partial\Pi^i$ at $t = \tau^1$.

We must show that once in Π^f, we remain there for all time. For $\Pi^f = \Pi_{III}$, we claim Π_{III} is invariant because under control M^{III}, along $p_2 = p_1/b_2$, $\dot{p}_2 = 0$, and $\dot{p}_1 = 1$, and along the $p_2 = 0$ axis, $\dot{p}_1 = M_2 p_1 + 1 > 0$. Therefore for $\Pi^f = \Pi_{III}$, once the costate flow enters Π^f, it never leaves. For $\Pi^f = \Pi_I$, the flow may approach Π_I from either Π_{II} or Π_{IV}.

In the case $\Pi^i = \Pi_{II}$, the system is governed by

$$\begin{pmatrix} \dot{p}_1 \\ \dot{p}_2 \end{pmatrix} = \begin{pmatrix} (M_1 + M_2) p_1 - M_2 b_2 p_2 + 1 \\ -p_1 + b_2 p_2 \end{pmatrix}. \tag{15}$$

From the above analysis we have shown that $p_2 > 1/M_2 b_2$ when the boundary is traversed. For such p, $\dot{p}_1 < (M_1 + M_2) p_1 < 0$. Moreover, along the line $p_2 = 1/M_2 b_2$, $\dot{p}_2 = -p_1 + 1/M_2 > 0$ for $p_1 < 0$. Therefore, if we enter Π_I from Π_{II}, we remain in it for all time. Finally, if we enter Π_I from Π_{IV}, from the above analysis, $p_1 < -1/M_1$, and $p_2 > p_1/b_2$. In this case $\dot{p}_1 = (M_1 + M_2) p_1 - M_2 b_2 p_2 + 1 < M_1 p_1 + M_2 p_2 - M_2 p_1 + 1 < -1 + 1 = 0$.

We are now ready to prove that the synthesis is optimal.

Proof (Proof of Theorem 3) We must show that the controlled trajectory satisfies all conditions outlined in Section 6
For conditions (1) and (2): define $P^0 = \{x^f\}$ to be the lone zero-dimensional cell, and it will be of the second kind. Likewise $P^2 = \text{int } S$ will be made up of cells A, B, C, and D of the first kind. Note that $P^2 - (P^{i-1} \cup N) = \mathscr{A} \cup \mathscr{B} \cup \mathscr{C} \cup \mathscr{D}$. Finally, let $P^1 = \{\sigma^i\}_{i=I}^{IV}$ where the σ^i are 1-dimensional cells of the second kind. Since v is constant in each cell $\mathscr{A}, \mathscr{B}, \mathscr{C}, \mathscr{D}$, and each σ^i, it is continuous and continuously differentiable and can be extended as a continuously differentiable function into a neighborhood of each cell.
For condition (3):
(a) We begin by showing that each point of the 2-dimensional cells $\mathscr{A}, \mathscr{B}, \mathscr{C}$, and $\mathscr{D}$ has a unique trajectory passing through it. Since $v(x)$ is constant in each cell, uniqueness is given; and by Lemma 2, for each cell, there are no rest points of the system.
(b) By Lemma 3 we show that the initial trajectories from regions $\mathscr{A}, \mathscr{B}, \mathscr{C}$, and $\mathscr{D}$ "pierce" their corresponding σ^i, and by Lemma 4, the trajectory leaves the cell ($\mathscr{A}, \mathscr{B}, \mathscr{C}$, or $\mathscr{D}$) in finite time.
(c) By definition the trajectories in σ^i approach the zero dimensional cell, and by Lemma 2 there is no rest point along any of the σ^i.
(d) We do not have any of these cases.
For condition (4): We have defined a unique distinguished trajectory such that only two cells are traversed before reaching x^f.
For condition (5): This is shown in Proposition 2.
For condition (6): This is shown in Lemma 5.

Now that we know that the control is optimal, we can prove the total transit time result.

Proof (Proof of Theorem 4) First, we address points in region $\mathscr{D}$. By Theorem 3, the control M^{II} for $0 \leq t \leq \tau$ and M^{III} for $\tau < t \leq t^*$ exists and is optimal. If we can find s and t such that $\phi_t^{M^{II}}(x^i) = \phi_s^{M^{III}}(x^f)$. Then the total transit time is $t^* = t - s$.

For $M = M^{II}$ we have the system

$$\begin{aligned} \frac{dx_1}{dt} &= -M_2 x_1 + x_2 + x_{np}, \quad x_1(0) = x_1^i, \\ \frac{dx_2}{dt} &= M_2 b_2 x_1 - b_2 x_2, \quad x_2(0) = x_2^i. \end{aligned} \tag{16}$$

We solve $\dot{x} = \mathbf{A}x + (x_{np}, 0)^T$ by variation of parameters:

$$x(t) = e^{t\mathbf{A}} x^i + e^{t\mathbf{A}} \int_0^t e^{-\tau \mathbf{A}} \begin{pmatrix} x_{np} \\ 0 \end{pmatrix} d\tau,$$

where, letting $a = (b_2 + M_2)$ the fundamental matrix solution is

$$e^{t\mathbf{A}} = \frac{1}{a} \begin{pmatrix} b_2 + e^{-at} M_2 & 1 - e^{-at} \\ b_2 M - b_2 e^{-at} M_2 & b_2 e^{-at} + M_2 \end{pmatrix}. \tag{17}$$

We have

$$e^{t\mathbf{A}}\int_0^t e^{-\tau\mathbf{A}}\begin{pmatrix}x_{np}\\0\end{pmatrix}d\tau = a^{-2}\begin{pmatrix}(M-e^{-at}M+ab_2t)\,x_{np}\\ b_2e^{-at}M_2\,(1+e^{at}(at-1))\,x_{np}\end{pmatrix}.$$

Therefore, the complete solution, after simplification is

$$\begin{aligned}x_1(t) &= a^{-2}\big(M_2x_{np}+a(x_2^i+b_2(x_1^i+tx_{np}))+(aM_2x_1^i-ax_2^i-M_2x_{np})e^{-at}\big),\\ x_2(t) &= a^{-2}\big(M_2(-b_2x_{np}+a(x_2^i+b_2(x_1^i+tx_{np})))+b_2(a(-M_2x_1^i+x_2^i)+M_2x_{np})e^{-at}\big).\end{aligned} \tag{18}$$

Next, for $M = M^{III}$, which is the solution along σ^{III}, we have (noting that we may solve backwards from x^f).

$$\begin{aligned}\frac{dy_1}{ds} &= y_2+x_{np}, \quad y_1(0)=x_1^f,\\ \frac{dy_2}{ds} &= -b_2y_2, \quad y_2(0)=x_2^f.\end{aligned} \tag{19}$$

By direct integration, the solution is

$$\begin{aligned}y_1(s) &= x_{np}s+\frac{x_2^f}{b_2}(1-e^{-b_2s})+x_1^f,\\ y_2(s) &= x_2^fe^{-b_2s}.\end{aligned} \tag{20}$$

Now, we solve $x_1(t)=y_1(s)$ and $x_2(t)=y_2(s)$ for s and t:

$$\begin{aligned}a^{-2}\big(Mx_{np}+a(x_2^i+b_2(x_1^i+tx_{np}))+(aMx_1^i-ax_2^i-Mx_{np})e^{-at}\big)&\\ = x_{np}s+\frac{x_2^f}{b_2}(1-e^{-b_2s})+x_1^f,& \quad (21)\\ a^{-2}\big(M(-b_2x_{np}+a(x_2^i+b_2(x_1^i+tx_{np})))+b_2(a(-Mx_1^i+x_2^i)+Mx_{np})e^{-at}\big)&\\ = x_2^fe^{-b_2s}.& \quad (22)\end{aligned}$$

Solving for e^{-b_2s} in equation (22) as a function of t yields

$$e^{-b_2s} = (x_2^fa^2)^{-1}\big(M(-b_2x_{np}+a(x_2^i+b_2(x_1^i+tx_{np})))+b_2(a(-Mx_1^i+x_2^i)+Mx_{np})e^{-at}\big), \tag{23}$$

which we can substitute into equation (21) and upon simplification we get

$$t^* = t-s = \frac{x_1^f-x_1^i}{x_{np}}+\frac{x_2^f-x_2^i}{b_2x_{np}}. \tag{24}$$

Therefore, if a solution of $x_1(t)=y_1(s)$ and $x_2(t)=y_2(s)$ exists, equation (24) is valid. By Lemma 4, this solution exists for all initial points $x^i\in\mathscr{D}$.

Now we address the case where $x^i\in\mathscr{A}$. By Theorem 3, the control M^{IV} for $0\le t\le\tau$ and M^{IV} for $\tau<t\le t^*$ exists and is optimal. If we can find s and t such that $\phi_t^{M^{IV}}(x^i)=\phi_s^{M^{III}}(x^f)$. Then the total transit time is $t^*=t-s$.

For $M = M^{IV}$ we have the system

$$\begin{aligned}\frac{dx_1}{dt} &= -M_1 x_1 + x_2 + x_{np}, \quad x_1(0) = x_1^i, \\ \frac{dx_2}{dt} &= -b_2 x_2, \quad x_1(0)x_2(0) = x_2^i.\end{aligned} \tag{25}$$

Again, we solve $\dot{x} = \mathbf{A}x + (x_{np}, 0)^T$ by variation of parameters. This time the fundamental matrix solution is

$$e^{tA} = \begin{pmatrix} e^{-M_1 t} & \frac{e^{-b_2 t} - e^{-M_1 t}}{-b_2 + M_1} \\ 0 & e^{-b_2 t} \end{pmatrix}$$

and then

$$e^{t\mathbf{A}} \int_0^t e^{-\tau\mathbf{A}} \begin{pmatrix} x_{np} \\ 0 \end{pmatrix} d\tau = \left(\frac{e^{-M_1 t}\left(-1 + e^{M_1 t}\right) z}{M_1}, 0 \right).$$

Therefore the complete solution after simplification is

$$\begin{aligned}x_1(t) &= \frac{e^{-b_2 t} x_2^i}{-b_2 + M_1} + \frac{x_{np}}{M_1} + e^{-M_1 t}\left(x_1^i + \frac{x_2^i}{b_2 - M_1} - \frac{x_{np}}{M_1} \right), \\ x_2(t) &= e^{-b_2 t} x_2^i.\end{aligned} \tag{26}$$

Using the solution for y from above, as before we must solve $x_1(t) = y_1(s)$ and $x_2(t) = y_2(s)$ for s and t. But $x_2(t) = y_2(t)$ implies

$$e^{-b_2 t} x_2^i = e^{-b_2 s} x_2^f,$$

which, upon simplification, gives

$$t - s = \ln(x_2^i / x_2^f).$$

By Lemma 4, this solution exists for all initial points $x^i \in \mathscr{A}$.

4 Application to Cryobiology

Here we present an application of our theory to the field of cryobiology. In order for cells and tissues to be successfully cryopreserved, cells and tissues must be equilibrated with high concentrations of cryoprotective agents such as glycerol or dimethyl sulfoxide [Mazur(1970)]. It has been shown that these chemicals have toxic effects at high concentrations [Fahy et al(2004)Fahy, Wowk, Wu, and Paynter]. Therefore it is of interest to describe mathematically an optimal protocol for the addition (and subsequent removal) of these cryoprotectants in which exposure time is minimized.

As an example, consider the two solute system containing sodium chloride and glycerol with associated extracellular molalities M_1 and M_2, where sodium chloride and glycerol are asumed membrane impermeable and permeable, respectively. For simplicity we normalize concentrations such that isosmolality is the unitary concentration, and suppose that the maximal salt concentration available is 10 times isosmotic, and the maximal glycerol concentration is 20 times isosmotic. If x_1, x_2 and

x_{np} are the normalized water volume, intracellular moles of permeating and non-permeating solutes, respectively, then the water and solute concentration (w_1 and w_2) inside the cell is given by the solution of

$$\begin{aligned} w_1' &= -M_1 - M_2 + \frac{x_{np} + w_2}{w_1}, \\ w_2' &= b(M_2 - \frac{w_2}{w_1}), \end{aligned}$$

which we reparametrize to

$$\begin{aligned} \dot{x}_1 &= -(M_1 + M_2)x_1 + x_{np} + x_2, \\ \dot{x}_2 &= b(M_2 x_1 - x_2). \end{aligned} \tag{27}$$

Let us suppose we have the initial condition $x^i = (x_1(0), x_2(0)) = (1, 0)$ corresponding to an isosmotic salt solution and a desired final condition $x^f = (x_1(t^*), x_2(t^*))) = (1, 10)$ corresponding to a concentration of glycerol roughly equivalent to 3 mol/kg dissolved in an isotonic salt solution. Finally, suppose that $b = 1$ (which is a physically realistic value [Benson(2009)]).

We may check (numerically or graphically) that this combination of initial and final conditions and maximal and minimal concentrations corresponds to the case where $x^i \in \mathscr{D}$. Thus the optimal control will be M^{II} on $t \in (0, \tau)$ and M^{III} on $t \in (\tau, t^*)$.

Thus for $t \in (0, \tau)$ system (27) becomes

$$\begin{aligned} \dot{x}_1 &= -20x_1 + x_2 + 1, \\ \dot{x}_2 &= 20x_1 - x_2. \end{aligned} \tag{28}$$

with initial conditions $x_1(0) = 1$ and $x_2(0) = 0$. For $t \in (\tau, t^*)$ system (27) becomes

$$\begin{aligned} \dot{y}_1 &= 1 + y_2, \\ \dot{y}_2 &= y_2. \end{aligned} \tag{29}$$

Because $x(\tau)$ is determined by the intersection of the solution of system (28) forward in time from x^i and the solution of system (29) backward in time from x^f, we have the final condition $x^f = (y_1(t^*), y_2(t^*))$.

The switching time τ may then be determined from the simultaneous solution of equations (21) and (22). It is possible to express the solution using special functions; but, for simplicity, we solve numerically for τ. By Theorem 4,

$$\begin{aligned} t^* &= \frac{x_1^f - x_1^i}{x_{np}} + \frac{x_2^f - x_2^i}{b_2 x_{np}} \\ &= \frac{1-1}{1} + \frac{10-1}{1} \\ &= 9. \end{aligned}$$

It remains to determine the switching time τ and final time t^* in un-transformed time, namely $q(\tau)$ and $q(t^*)$. These values are given by the integrals

$$q(\tau) = \int_0^\tau x_1(\xi)\, d\xi \tag{30}$$

and

$$q(t^*) = \int_0^9 x_1(\xi)\,d\xi = \int_0^\tau x_1(\xi)\,d\xi + \int_\tau^9 x_1(\xi)\,d\xi. \tag{31}$$

Because we have an exact solution for the x_1 variable given in displays (18) and (20), we may directly calculate the integral of x_1 on both $t \in (0,\tau)$ and $t \in (\tau, t^*)$ yielding the switching and final untransformed times.

5 Conclusion

We have presented an analysis of a multi-solute extension of a previously published general model of cell volume and concentration regulation and we have extended the local stability result in the case $n = 2$ presented in a previous work to global asymptotic stability in the case $n \geq 2$. Moreover we have given an application of this result in control theory, and provided a complete synthesis of an optimal control in a commonly encountered two solute biological system. Finally, we have demonstrated that for initial points in two special cases, there are explicit and simple formulas that define the total transit time. Although the implementation of an optimal control scheme such as this in the biological setting is dependent on the sensitivity to parameters, as long as it can be verified that the initial point is in one of the defined regions above, this optimal control formulation gives an estimate of the minimal transport time that can be achieved. Because of this, one can determine how much engineering, biophysics, or biology is worth undertaking to achieve more optimal protocols.

Acknowledgements Funding for this research was provided by the University of Missouri, NSF grant NSF/DMS-0604331 (C.Chicone PI), NIH grants U42 RR14821 and 1RL 1HD058293 (J.K. Critser PI), and the National Institute of Standards and Technology National Research Council postdoctoral associateship.

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6 Appendix

This is from Chapter 4, Section 12, Paragraph 45 of *Mathematical Methods of Optimal Control*, V.G. Boltyanskii, 1971. We add subitems to make some statements more concrete.

> We first introduce the concept of *regular synthesis* for the system $\dot{x} = f(x,u)$ for which the continuity of the derivatives $\partial f^i/\partial x^j$ and $\partial f^i/\partial u^k$ will not be assumed. Suppose that a piecewise smooth set N of dimension $\leq n-1$, piecewise smooth sets
>
> $$P^0 \subset P^1 \subset \ldots \subset P^{n-1} \subset P^n = S,$$
>
> and a function $v : S \to \mathscr{A}$ are given. We will say that the sets P^i and the function v realize a *regular synthesis* for $\dot{x} = f(x,u)$ in the region S if the following conditions are satisfied:
>
> 1. The set P^0 contains the point $a = x_1$ but does not have limiting points in the open set S. Each component of the set $P^i - (P^{i-1} \cup N)(i = 1,2,\ldots,n)$ is a smooth $i-$dimensional manifold in S; these components will be called *i-dimensional cells*. The points of the set P^0 will be called zero-dimensional cells. The function $v(x)$ is continuous and continuously differentiable on each cell and can be extended as a continuously differentiable function into a neighborhood of the cell.
> 2. All cells are grouped into cells of the first and second kind. All $n-$dimensional cells are cells of the first kind, all zero-dimensional cells are cells of the second kind.
> 3. (a) If σ is an $i-$dimensional cell of the first kind, then each point of this cell has a unique trajectory of the equation
>
> $$\dot{x} = f(x, v(x)) \qquad (32)$$
>
> passing through it.

(b) There exists an $(i-1)-$dimensional cell $\Pi(\sigma)$ such that each trajectory of the system (32) traversing the cell σ leaves this cell after a finite time, arrives at the cell $\Pi(\sigma)$ at a nonzero angle, and approaches the latter with a nonzero phase velocity.

(c) If σ is a one dimensional cell of the first kind, then it is a piece of a phase trajectory of system (32) approaching a zero-dimensional cell $\Pi(\sigma)$ with a nonzero phase velocity.

(d) If σ is an $i-$dimensional cell of the second kind distinct from the point a, then there exists an $(i+1)-$dimensional cell $\Sigma(\sigma)$ of the first kind such that from any point of the cell σ there emanates a unique trajectory of system (32) traversing the cell $\Sigma(\sigma)$; moreover, the function $v(x)$ is continuous and continuously differentiable on $\sigma \cup \Sigma(\sigma)$.

4. The above conditions make it possible to continue the trajectories of 32 from cell to cell: from the cell σ to the cell $\Pi(\sigma)$ if $\Pi(\sigma)$ is of the first kind, and from the cell σ to the cell $\Sigma(\Pi(\sigma))$ if $\Pi(\sigma)$ is of the second kind. It is required that each such trajectory traverse only a finite number of cells (that is, each such trajectory "pierces" only a finite number of cells of the second kind). Moreover, each such trajectory terminates at the point a. The above trajectories will be called "distinguished" trajectories. Thus, a single distinguished trajectory (leading to the point a) emanates from every point of the set $G-N$. It is also required that a (possibly non-unique) trajectory of system (32) leading to the point a emanates from every point of the set N. These will also be called "distinguished" trajectories.
5. All distinguished trajectories satisfy the maximum principle.
6. The value of the transition time from the point x_0 to the point a, calculated along distinguished trajectories (terminating at the point a), is a continuous function of the initial point x_0. (In particular, if several distinguished trajectories emanate from the point $x_0 \in N$, then the value of the transition time is the same for these trajectories.)

Theorem 5 *If a regular synthesis is realized in S for $\dot{x} = f(x,u)$ (assuming the existence of continuous derivatives $\frac{\partial f^i}{\partial x^j}$ and $\frac{\partial f^i}{\partial u^k}$), then all distinguished trajectories are optimal (in S).*