Optimal Carbon Source Switching Strategy for the Production of PHA Copolymers

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During polymerization in a nongrowing cell population of Ralstonia eutropha, alternating between two different carbon sources (fructose and fructose/valeric acid) could lead to the production of block copolymers consisting of blocks of homo-poly-3-hydroxybutyrate (PHB) and polyhydroxybutyrate-co-valerate (PHBV) copolymer. The problem of finding the optimal number of carbon source switches and corresponding switching times that maximize the final concentration of diblock copolymers (PHB-PHBV and PHBV-PHB) was addressed. It was mathematically formulated in the mixed-integer nonlinear programming (MINLP) framework, which allows the decomposition of the original problem into the primal and master problems. The primal problem corresponds to the original problem for a fixed number of carbon source switches, whereas the master problem consists of finding the number of carbon source switches that maximizes the optimum solutions of all possible primal problems. The global optimum was obtained for 39 carbon source switches. It corresponds to a mass fraction of 50.6% of final diblock copolymer concentration over the final total polymer concentration.

Introduction

Polyhydroxyalkanoates (PHA) are biodegradable polyesters that are synthesized by many bacteria under imbalanced growth conditions. They serve as an intracellular storage product for carbon and energy (Lee, 1996; Steinbuchel, 1991; Anderson and Dawes, 1990), which can be utilized to support growth when an extracellular carbon source is absent. Polyhydroxybutyrate (PHB) is the longer known of these biological polyesters and has attracted significant commercial interest. It is biodegradable when discarded in the environment and biocompatible when implanted in the body. However, it has been shown that many additional (R)-3-hydroxyacids can also serve as building blocks for these polyesters. Thus, many different types of homopolymers, copolymers and block copolymers can be synthesized. Their physical properties are determined by the nature and sequence of the monomer units present in the polymer chains.

Cellular regulation mechanisms are responsible for temporal separation of polymer synthesis from cell growth in which cells mainly multiply and reproduce themselves. Under adverse (such as nitrogen limited) growth conditions, the flux of metabolites can be effectively directed from pathways leading to growth products, such as proteins, to the synthesis of storage polymers, such as PHAs.

The PHA polymerization step in *Ralstonia eutropha* is catalyzed by a synthase enzyme, which elongates the growing polymer chains by successively adding monomer units. Due to the fact that the synthase enzyme is not very substrate specific, different types of monomers can be incorporated in the actively elongating polymer chain, leading to the formation of copolymers. The intracellular pool of monomers can be manipulated by exposing the cells to different extracellular carbon sources that feed different precursor molecules into the monomer pool via different degradation pathways. Therefore, the type of copolymers or homopolymers synthe-

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sized can be manipulated by switching between the extracellular carbon source.

If the available carbon source is only fructose, then the cellular metabolism leads to the production of intracellular (R)-3-hydroxybutyrate (HB) monomer units. In constrast, if both fructose and valeric acid are available as carbon sources, the (R)-3-hydroxy valerate (HV) monomer units are added to the monomer pool as well. The incorporation of just HB monomer units to actively growing polymer chains leads to the production of PHB, whereas the simultaneous addition of HB and HV monomer units results in the formation of PHBV copolymer chains. We have previously shown that the morphology of polymer granules can be manipulated by exposing cells alternatively to different carbon sources (Kelley and Srienc, 1999). Under such conditions, layers of different polymer types can be synthesized on top of each other by switching the nature of the carbon source that is utilized by the cells. It is now of interest whether such carbon source switching can be carried out in a way that amounts of block copolymers would be synthesized in quantities that are technologically useful. In order to investigate the possibility of block copolymer formation with the implementation of a carbon source switching strategy, it is necessary to be able to accurately predict the microstructure of the produced polymer chains.

For this purpose, in our previous work (Mantzaris et al., 2001a), we have developed a mathematical model that can predict the dynamics of the intracellular bulk monomer concentration, as well as those of the molecular weight distributions of the active and inactive chains. According to this model, the molecular weight distribution of the polymer chains depends on three rates: (a) the rate by which the polymerization process is initiated; (b) the elongation rate by which the synthase enzyme catalyzing the polymerization step adds monomer units to the actively growing polymer chains; and (c) the termination rate that determines the rate by which the active chains stop elongating, thus becoming inactive. The steady-state version of the model in conjunction with available experimental data was used in order to obtain important information not directly obtainable from experiment. In particular, we computed the steady-state active chain molecular weight distribution, the termination rate as a function of polymer molecular weight, the initiation rate, as well as the corresponding model parameter values that appear in the model equations. The computations were performed for both cases of PHB and PHBV formation.

Furthermore, we also analyzed the transient behavior of the system for the simplest case of constant elongation rate (Mantzaris et al., 2001b). We have used the model parameter values that were computed from the steady-state analysis and analytically evaluated the time evolution of both the active and inactive chain molecular weight distributions. The analytical solution was subsequently used in order to study the entire carbon source switching process for a wide array of number of carbon source switches. The final (after N carbon source switches) molecular weight distributions and corresponding concentrations of all different types of polymers (block copolymers and homopolymers) were computed. It is possible to control the duration of each stage of the carbon source switching process to any desired values. However, these computations were based on the assumption that the duration of each PHBV producing stage is exactly half the duration of each PHB producing stage. The results indicated that under this assumption, the final concentrations of diblock (PHB-PHBV or PHBV-PHB) and triblock (PHBV-PHB-PHBV or PHB-PHBV-PHB) copolymers exhibit a maximum. In the case of diblock copolymers, the value of the maximum was 50% of the total polymer produced, and was achieved for $N = 39$ carbon source switches. The corresponding switching times were approximated 30 and 60 min for each PHBV and PHB (respectively) producing stage. In the case of triblock copolymers, the value of the maximum was 28.5% of the total polymer produced, and was achieved for $N = 90$ carbon source switches. The corresponding switching times were approximately 12.5 and 25 min for each PHBV and PHB (respectively) producing stage.

The question that naturally arises from these results is: Are those values of the final concentrations of di- and triblock copolymers the maximum values that can possibly be achieved? Or, in other words, is there another set of number N of carbon source switches and corresponding N switching times that can yield a higher value than the one achieved for $N = 39$ (or $N = 90$) carbon source switches and under the assumption that the duration of each PHBV producing stage is half the duration of each PHB producing stage?

In this work, we address this question for the case of diblock copolymers. More specifically, we consider the problem of finding the optimal number of carbon source switches and corresponding switching times that will maximize the final concentration of produced diblock copolymers. We chose the diblock copolymer case as an example that will illustrate the general methodology that should be used in order to address similar optimization problems, such as maximizing the final triblock copolymer concentration, or the sum of final di- and triblock copolymer concentrations, or the final concentration of chains with a particular microstructure of interest (such as diblock copolymer chains with the same number of PHB and PHBV monomer units) and so on. Initially, the mathematical model that was developed in order to describe the dynamics of the active and inactive molecular weight distributions is briefly presented. Then, the carbon source switching process is described. The optimization problem is mathematically formulated and analyzed under consideration in the context of the mixed-integer nonlinear programming (MINLP) framework. Finally, the solution method and the results are presented.

Mathematical Model

The development of the model has been described in detail in our previous work (Mantzaris et al., 2000a). For the sake of clarity, the key aspects and assumptions of the model are summarized here again.

Consider a population of cells of *Ralstonia eutropha* which occupies a fixed biovolume V. Each cell of the population contains a fixed number of granules. We assume that the entire cell population is homogeneous with respect to the polymerization processes, that is, each cell in the population behaves exactly like the population average cell. Based on experimental observations that were discussed in our previous work, we also assume that during the entire polymerization process, the intracellular bulk monomer concentration does not change. Let $A(x,t)dx$ and $I(x,t)dx$ be the number of active and inactive (respectively) chains per unit biovolume, which at time t have molecular weight between x and $x + dx$. Let also $R_{el}(x)$ and $R_t(x)$ denote, respectively, the single-chain elongation rate and termination rate of an active polymer chain with molecular weight x. Finally, r_p and r_i are, respectively, the rate of monomer production per unit volume of biomass from upstream metabolic processes, and the rate of bulk monomer loss per unit volume of biomass due to initiation of the polymerization process. Then, the dynamics of $A(x,t)$, $I(x,t)$ are described by the following set of equations

$$\frac{\partial A(x,t)}{\partial t} + \frac{\partial}{\partial x}\left[R_{el}(x)A(x,t)\right] + R_t(x)A(x,t) = 0 \quad (1)$$

$$\frac{\partial I(x,t)}{\partial t} = R_t(x)A(x,t) \quad (2)$$

where $x \in [x_{\min}, x_{\max}]$ and $t \in [0,\infty]$. The boundary condition was shown to be

$$x_{\min} R_{el}(x_{\min}) A(x_{\min}, t) = N_A r_i \quad (3)$$

where N_A is the Avogadro number. The steady-state analysis has shown that the rate of initiation is related to the rate of monomer production through the equation

$$r_i = x_{\min} \frac{r_p}{\overline{MW}} \quad (4)$$

where $\overline{MW}$ is the average molecular weight of the steady-state overall (active and inactive) chains which was experimentally measured.

Moreover, we assume that the single-chain elongation rate is a constant independent of the polymer molecular weight x, that is

$$R_{el}(x) = E_1 \quad (5)$$

The steady-state analysis of the system has revealed that for this elongation rate expression, the single-chain termination rate exhibits a maximum after it slowly decreases. This type of behavior was shown to be accurately described by the following equation

$$R_t(x) = \frac{T_1(x - x_{\min})}{T_2 + (x - x_{\min}) + T_3(x - x_{\min})^2} \quad (6)$$

where T_1, T_2 and T_3 are positive constants.

In order to bring the equations in a more convenient form, we first define the dimensionless molecular weight and the dimensionless time as follows

$$y = \frac{x - x_{\min}}{x_{\max} - x_{\min}} \quad (7)$$

$$\tau = \frac{E_1}{(x_{\max} - x_{\min})} t \quad (8)$$

We also set

$$A(x,t) = A(y,\tau) \quad (9)$$

$$I(x,t) = I(y,\tau) \quad (10)$$

Finally, we define the following dimensionless parameters

$$t_1 = \frac{T_1(x_{\max} - x_{\min})}{E_1} \quad (11)$$

$$t_2 = \frac{T_2}{x_{\max} - x_{\min}} \quad (12)$$

$$t_3 = T_3(x_{\max} - x_{\min}) \quad (13)$$

Using Eqs. 5 and 6 for the elongation and termination rate, respectively, the transformation defined by Eqs. 7–10 and the definitions Eqs. 11–13 for the dimensionless model parameters t_1, t_2, t_3, the population balance equations for the active and inactive polymer chains (Eq. 1 and Eq. 2), respectively, become

$$\frac{\partial A(y,\tau)}{\partial \tau} + \frac{\partial A(y,\tau)}{\partial y} + \frac{t_1 y}{t_2 + y + t_3 y^2} A(y,\tau) = 0 \quad (14)$$

$$\frac{\partial I(y,\tau)}{\partial \tau} = \frac{t_1 y}{t_2 + y + t_3 y^2} A(y,\tau) \quad (15)$$

where $y \in [0,1]$ and $\tau \in [0,\infty]$. The boundary condition (Eq. 3) takes the form

$$A(0,\tau) = \frac{r_i N_A}{E_1 x_{\min}} \quad (16)$$

The initial conditions for the active and inactive population balance Eqs. 14 and 15 are expressed by the equations

$$A(y,0) = A_o(y) \quad (17)$$

$$I(y,0) = I_o(y) \quad (18)$$

Notice that Eqs. 14, 15, and 16 contain five model parameters: the initiation rate r_i, the single-chain elongation rate E_1 and the three dimensionless termination rate parameters t_1, t_2, and t_3. The values of these parameters calculated in Mantzaris et al. (2000a) are given in Table 1, for both cases of PHBV and PHB formation. The values of the two rates of monomer production (r_p), which are related to the corresponding rates of initiation through Eq. 4 are also given in Table 1.

Table 1. Model Parameter Values

Parameter (Dimensional)	Copolymer (PHBV)	Polymer (PHB)
E_1	1,327,400 g/mol/h	618,065 g/mol/h
r_i	17.88 g/L/h	15.64 g/L/h
r_p	164 g/L/h	83 g/L/h
T_1	7.041 L/h	2.823 L/h
T_2	104,439 g/mol	38,370 g/mol
T_3	7.229×10^{-7} mol/g	5.388×10^{-7} mol/g
Parameter (Dimensionless)	**Copolymer (PHBV)**	**Polymer (PHB)**
t_1	20.073	17.283
t_2	2.760×10^{-2}	1.014×10^{-2}
t_3	2.735	2.039

Carbon Source Switching Process

Processing time

In this section we consider and study the process which starts at time $T = 0$, ends at a final time $T = T_f$ (processing time), and consists of N carbon source switches in between. It is assumed that at $T = 0$, there is no polymer present in the system. Thus, $T = 0$ is the point in time where the polymerization process is turned on. Moreover, it has been experimentally established (Kelley and Srienc, 1999) that the total polymer concentration for both polymers (PHB or PHBV) follows a linear (constant rate) increase with time over a large period of time, which depends on the type of polymer produced (PHB or PHBV). After that point in time, the concentration vs. time profiles start to become flat. This behavior can most probably be attributed to a saturation effect due to the increase of biovolume by the accumulated polymer. Therefore, when the polymer concentration stops to increase linearly with time, our mathematical model, which is based on the assumption of constant biovolume, is no longer valid. The experimental measurements indicated that in both cases of PHB or PHBV production, the constant synthesis rates were observed until the total polymer content becomes greater than 70% of the cell dry weight. We have used these observations to implicitly define the final time of the process T_f, as the time where the maximum possible amount of polymer has been produced (70% of cell dry weight) under the assumption of constant biovolume, thus ensuring the validity of the proposed mathematical model.

Let t_i, $i = 1, 2, \ldots, N$ denote the N switching times. We define the term "switching time" as the time elapsed since the previous carbon source switch, or the time length of each stage of the carbon source switching process. Thus, the ith stage of the process takes place in the time interval $[0, t_i]$, or in dimensionless form: $[0, \tau_i]$, where τ_i is the corresponding dimensionless switching time (see Eq. 8). With this convention, the total time that has elapsed after i carbon source switches, since the beginning of the entire process ($T = 0$) is then given by: $T_i = \Sigma_{j=1}^{i} t_j$. The total concentration of polymer that has been produced at the end of the process ($T = T_f$), after N carbon source switches have been performed, can then be expressed as: $P = \Sigma_{i=1}^{N} r_p^i \cdot t_i$ or by using the definition (Eq. 8) of the dimensionless time:

$$P = (x_{\max} - x_{\min}) \cdot \sum_{i=1}^{N} \frac{r_p^i}{E_1^i} . \tau_i ,$$

where r_p^i and E_1^i represent the rate of monomer production and polymer elongation rate, respectively, at the ith stage of the switching process (see Table 1) and τ_i is the corresponding dimensionless switching time. As explained above, at the end of the process, the total polymer produced is taken to be equal to 70% of the cell dry weight. If we denote by CDW the initial (when no polymer is present) cell dry weight density which was experimentally measured to be 1.36 kg/L biovolume, the following algebraic constraint has to be satisfied

$$\frac{P}{P + CDW} = 0.7 \tag{19}$$

or after substituting the expression for P into (Eq. 19)

$$\sum_{i=1}^{N} \frac{r_p^i}{E_1^i} \cdot \tau_i = \frac{7}{3} \frac{CDW}{(x_{\max} - x_{\min})} \tag{20}$$

Keeping track of polymer chains with different structure

Let us now examine the switching process in detail. It is assumed that the process begins with the production of PHBV chains. At the point of the first carbon source switch, the system contains only PHBV in both active and inactive forms. At the time when the second switch is performed, the system consists of two subpopulations: (a) the active and inactive PHB-PHBV diblock copolymer chains which originated from the population of PHBV homopolymer chains that were active when the first switch was performed; and (b) the new population of active and inactive PHB homopolymer chains that were produced between the first and second carbon source switches. Similarly, at the time where the third switch is performed, the system consists of three subpopulations: (a) the active and inactive PHBV-PHB-PHBV triblock copolymer chains which originated from the population of PHB-PHBV diblock copolymer chains that were active when the second carbon source switch took place; (b) the active and inactive PHBV-PHB diblock copolymer chains which originated from the population of PHB homopolymer chains that were active when the second switch was performed; and (c) the new population of active and inactive PHB homopolymer chains that were produced between t_2 and t_3. Thus, after each carbon source switch, a new type of block copolymer chains is created. Since no block copolymer is being produced during the first stage of the process, after N carbon source switches, the system contains N-1 different types of block copolymers, homopolymer (PHB), and copolymer (PHBV). In the following, and in order to be consistent with the terminology that will be used, we will refer to PHB and PHBV chains as mono-block copolymer chains, despite the fact that PHB is a homopolymer and PHBV is not a block copolymer.

As has been explained in detail in our previous work (Mantzaris et al., 2001b), the molecular weight distributions of the active and inactive mono-block copolymers $A_1^i(y, \tau_i)$, $I_1^i(y, \tau_i)$, that are produced during the ith stage of the process depend only on the ith (current) dimensionless switching time τ_i. Their dynamics are only influenced by the boundary condition (Eq. 3) and are expressed by the following equations

$$A_1^i(y, \tau_i) = \begin{cases} D^i \cdot \left(y + \dfrac{1 - \sqrt{1 - 4 t_2^i t_3^i}}{2 t_3^i} \right)^{\frac{-t_1^i}{2 t_3^i} + \frac{t_1^i}{2 t_3^i \sqrt{1 - 4 t_2^i t_3^i}}} \\ \quad \cdot \left(y + \dfrac{1 + \sqrt{1 - 4 t_2^i t_3^i}}{2 t_3^i} \right)^{\frac{-t_1^i}{2 t_3^i} - \frac{t_1^i}{2 t_3^i \sqrt{1 - 4 t_2^i t_3^i}}} , \\ \qquad\qquad\qquad\qquad\qquad\qquad \tau_i > y \\ 0, \qquad\qquad\qquad\qquad\qquad\qquad \tau_i \le y \end{cases} \tag{21}$$

$$I_1^i(y,\tau_i)=\begin{cases}\dfrac{t_1^i y}{t_2^i+y+t_3^i y^2}\cdot A_1^i(y,\tau_i)\cdot(\tau_i-y), & \tau_i>y\\ 0, & \tau_i\le y\end{cases}\tag{22}$$

where

$$D^i=\frac{N_A r_i^i}{E_1^i x_{\min}}\left(\frac{1-\sqrt{1-4t_2^i t_3^i}}{2t_3^i}\right)^{\frac{-t_1^i}{2t_3^i}-\frac{t_1^i}{2t_3^i\sqrt{1-4t_2^i t_3^i}}}\cdot\left(\frac{1+\sqrt{1-4t_2^i t_3^i}}{2t_3^i}\right)^{\frac{t_1^i}{2t_3^i}+\frac{t_1^i}{2t_3^i\sqrt{1-4t_2^i t_3^i}}}\tag{23}$$

and r_i^i, E_1^{i}, t_1^i, t_2^i, t_3^i denote the values of the initiation rate, the elongation rate, and the three dimensionless termination rate model parameters that correspond to the ith stage of the process.

On the other hand, the molecular weight distributions of active and inactive m-block copolymers $A_m^i(y,\tau_i)$, $I_m^i(y,\tau_i)$ $(m=2,\dots,N)$ produced at the ith $(N\ge i\ge m)$ stage of the switching process depend on the $m-1$ previous dimensionless switching times, as well as the current dimensionless switching time τ_i. Moreover, $A_m^i(y,\tau_i)$ is the solution of Eq. 14 subject to the initial condition (Eq. 17) with: $A_o(y)=A_{m-1}^i(y,\ \tau_{i-1})$, that is, the molecular weight distribution of active $(m-1)$-block copolymers at the previous carbon source switch. Due to the fact that the inactive m-block copolymers that are produced during the ith stage of the process originate only from the termination of active m-block copolymers during the same stage, the initial condition for the inactive distribution is always zero. Based on these observations, $A_m^i(y,\tau_i)$, $I_m^i(y,\tau_i)$ $(m=2,\dots,N$ and $N\ge i\ge m)$ were shown to be expressed by the following equations

$$A_m^i(y,\tau_i)=\begin{cases}A_{m-1}^{i-1}(y-\tau_i,\tau_{i-1})\\ \quad\cdot\left(y-\tau_i+\dfrac{1-\sqrt{1-4t_2^i t_3^i}}{2t_3^i}\right)^{\frac{t_1^i}{2t_3^i}-\frac{t_1^i}{2t_3^i\sqrt{1-4t_2^i t_3^i}}}\\ \quad\cdot\left(y-\tau_i+\dfrac{1+\sqrt{1-4t_2^i t_3^i}}{2t_3^i}\right)^{\frac{t_1^i}{2t_3^i}+\frac{t_1^i}{2t_3^i\sqrt{1-4t_2^i t_3^i}}}\\ \quad\cdot\left(y+\dfrac{1-\sqrt{1-4t_2^i t_3^i}}{2t_3^i}\right)^{\frac{-t_1^i}{2t_3^i}+\frac{t_1^i}{2t_3^i\sqrt{1-4t_2^i t_3^i}}}\\ \quad\cdot\left(y+\dfrac{1+\sqrt{1-4t_2^i t_3^i}}{2t_3^i}\right)^{\frac{-t_1^i}{2t_3^i}+\frac{t_1^i}{2t_3^i\sqrt{1-4t_2^i t_3^i}}}, & \\ & \tau_i\le y\\ 0, & \tau_i>y\end{cases}\tag{24}$$

$$I_m^i(y,\tau_i)=\begin{cases}\dfrac{t_1 y}{t_2+y+t_3 y^2}\cdot\displaystyle\int_0^{\tau}A_m^i(y,\tau')d\tau', & \tau_i\le y\\ \dfrac{t_1 y}{t_2+y+t_3 y^2}\cdot\displaystyle\int_0^{y}A_m^i(y,\tau')d\tau', & \tau_i>y\end{cases}\tag{25}$$

for $m=2,\dots,N$. For $m=2$, the initial condition $A_{m-1}^i(y,\ \tau_{i-1})$ that appears in Eq. 24 is expressed by Eq. 21.

Since we are interested in optimizing the carbon source switching process for the production of the maximum possible concentration of diblock copolymers, we will hereafter concentrate on just this type of block-copolymers. The active and inactive diblock copolymer molecular weight distributions produced during the ith stage of the process are given by Eqs. 24 and 25, respectively, with: $A_{m-1}^{i-1}(y-\tau_i,\ \tau_{i-1})=A_1^{i-1}(y-\tau_i,\ \tau_{i-1})$ and $A_1^{i-1}(y-\tau_i,\ \tau_{i-1})$ expressed by Eq. 21. For all stages, except the last one (N^{th} stage), the active diblock copolymers produced at the ith stage of the process are always transformed into triblock copolymers during the subsequent stage. Thus, the final concentration of diblock copolymers will consist of the inactive chains that were produced during all diblock copolymer producing stages of the process (stage 2 up to stage N) and of the active chains produced during the Nth stage of the process. Therefore, the active chains represent only a very small fraction of the final diblock copolymer concentration. Due to this fact, we focused on the inactive chains produced during each stage of the process.

As explained above, the active and inactive diblock copolymer distributions produced at the ith stage of the process, depend only on the current and previous dimensionless switching times (τ_i, τ_{i-1}, respectively). By appropriately substituting Eqs. 21 into Eqs. 24 and 25 for $m=2$, we derived the mathematical formulae which give the concentration of inactive diblock copolymers produced during the ith stage of the process (first moment of the corresponding distribution) $C_2^i(\tau_{i-1},\ \tau_i)$ as an explicit function of the two dimensionless switching times. Due to their complexity and their dependence on the absolute and relative magnitude of the two dimensionless switching times, these formulae are given in Appendix A (Eqs. A12, A18 and A19). We have also derived (see Appendix A, Eqs. A13–A17) the corresponding formulae for the two first partial derivatives

$$\left(\frac{\partial C_2^i(\tau_{i-1},\tau_i)}{\partial\tau_{i-1}},\frac{\partial C_2^i(\tau_{i-1},\tau_i)}{\partial\tau_i}\right)$$

and the three second derivatives

$$\left(\frac{\partial^2 C_2^i(\tau_{i-1},\tau_i)}{\partial\tau_{i-1}^2},\frac{\partial^2 C_2^i(\tau_{i-1},\tau_i)}{\partial\tau_i^2},\frac{\partial^2 C_2^i(\tau_{i-1},\tau_i)}{\partial\tau_{i-1}\partial\tau_i}\right.$$
$$\left.\text{with }\frac{\partial^2 C_2^1(\tau_{i-1},\tau_i)}{\partial\tau_i\partial\tau_{i-1}}\right).$$

The mathematical properties of all these functions have also been analyzed in Appendix A.

Formulation of the Optimization Problem

Our objective is to maximize the final concentration of diblock copolymers. Therefore, the objective function f to be minimized is minus the sum of the concentrations of inactive diblock copolymers, produced at each stage of the process (from stage 2 p to stage N). The variables that are available for manipulation (optimization variables) are the number N of carbon source switches and the corresponding N dimensionless switching times τ_i, $i = 1, \ldots, N$. It is obvious that we cannot allow negative or zero dimensionless switching times. Thus, if we define by $\boldsymbol{\tau} = [\tau_1\, \tau_2\, \tau_3 \ldots \tau_N]^T$, the N-dimensional vector containing all dimensionless switching times, we must have that $\boldsymbol{\tau} \in \boldsymbol{T} = R_+^N$. The end of the process is defined as the time when the total polymer produced is equal to 70% of the total cell dry weight. Thus, Eq. 20 is an equality constraint that must be satisfied by the optimization variables. Moreover, there must be a minimum concentration of polymer that is (at least) produced during each stage of the process, in order for the stage to be of practical meaning. We chose this minimum polymer concentration to be 50 g/L. Since the polymer concentration produced at each stage of the process is given by $(x_{\max} - x_{\min}) \cdot (r_p^i / E_1^l) \cdot \tau_i$, and the ratio of r_p / E_1 is approximately the same for each stage of the process, we easily see that the minimum requirement for polymer production is satisfied for dimensionless switching times which are greater or equal to $\tau_{\min} = 0.1$. Therefore, we also demand that all dimensionless switching times are greater or equal to $\tau_{\min} = 0.1$. Since no diblock copolymer chains are produced during the first stage of the process, the minimum number N of carbon source switches is 2. Moreover, using the values of r_p, E_1 (see Table 1) and CDW (= 1.36 Kg/L), we can easily see that for $N > 65$, and in order to satisfy the equality constraint (20), at least one of the dimensionless switching times should be smaller than $\tau_{\min} = 0.1$. Thus, for $N > 65$, the equality and inequality constraints can not be simultaneously satisfied. Therefore, we should have: $2 \le N \le N_{\max} = 65$. Thus, we can state the optimization problem as follows:

Find the number N of carbon source switches $2 \le N \le N_{\max} = 65$ and corresponding N dimensionless switching times with $\tau_i \ge 1 \forall i = 1, \ldots, N$, that maximize the final (after N carbon source switches) diblock copolymer concentration.

Since one of the optimization variables (the number N of the carbon source switches) is an integer, while the rest (the N dimensionless switching times) are continuous and real variables, a natural mathematical formulation of the problem is within the mixed-integer nonlinear programming framework (MINLP) (Floudas, 1995).

Let us first substitute the optimization variable N with a new set of integer valued optimization variables: a vector $\boldsymbol{w}$ of $N_{\max} = 65$ 0 or 1 integer variables. The value of 1 (0) for the entry w^i of the vector $\boldsymbol{w}$ denotes existence (nonexistence) of the ith stage of the process. From the description of the carbon source switching process, it is obvious that the ith stage of the process cannot exist if the preceding $(i-1)$th stage does not exist as well. Thus, the vector $\boldsymbol{w}$ can only be of the form: $\boldsymbol{w} = [1\ 1 \ldots. 1\ 0\ 0 \ldots 0]^T$. This implies that if an entry of the vector $\boldsymbol{w}$ is equal to zero then: (a) all the following entries must also be zero and either (b) the previous entry is also equal to zero, or (c) the previous entry is the last one (1) entry of the vector. In more compact form: $\boldsymbol{w} \in \boldsymbol{W}$, where the space $\boldsymbol{W}$ is defined as

$$\boldsymbol{W} = \left\{ \begin{array}{l} \boldsymbol{w} \in \boldsymbol{W}, w^i = 0 \text{ or } 1 \ \textbf{s.t.:} \\ \\ \qquad w^i = 0 \Rightarrow \left\{ \begin{array}{l} (a)\ w^j = 0, j = i+1, \ldots, N_{\max} \\ \text{and either} \\ (b)\ w^{i-1} = 0 \\ \text{or} \\ (c)\ w^j = 1, j = 1, \ldots, i-1 \end{array} \right\} \end{array} \right.$$

Notice that this new set of integer optimization variables implicitly includes the old optimization variable N as the number of consecutive entries with value 1 in vector $\boldsymbol{w}$. Using this new set of optimization variables, we can mathematically express the objective function as follows

$$f(\boldsymbol{w}, \boldsymbol{\tau}) = -\sum_{i=2}^{N_{\max}} w^i \cdot C_2^i(\tau_{i-1}, \tau_i) \tag{26}$$

Moreover, the equality constraint (Eq. 20) takes the form

$$h(\boldsymbol{w}, \boldsymbol{\tau}) = (x_{\max} - x_{\min}) \cdot \sum_{i=1}^{N_{\max}} w^i \cdot \frac{r_p^i}{E_1^i} \cdot \tau_i - \frac{7}{3}\text{CDW} = 0 \tag{27}$$

Finally, the inequality constraints discussed above can be expressed as

$$g_i(w^i, \tau_i) = w^i \cdot (\tau_{\min} - \tau_i) \le 0, i = 1, 2, \ldots, N_{\max} \tag{28}$$

Based on the preceding analysis, we can mathematically formulate the optimization problem under consideration as follows:

$$\begin{array}{l} \min\limits_{\tau, w} f(\boldsymbol{w}, \boldsymbol{\tau}) \\ \text{subject to:} \\ h(\boldsymbol{w}, \boldsymbol{\tau}) = 0 \\ \text{and} \\ \boldsymbol{g}(\boldsymbol{w}, \boldsymbol{\tau}) \le 0 \\ \boldsymbol{w} \in \boldsymbol{W} \\ \boldsymbol{\tau} \in \boldsymbol{T} \end{array} \tag{29}$$

where f and h are the scalar functions defined by Eqs. 26 and 27, respectively, and $\boldsymbol{g}$ is the $N_{\max}$-dimensional vector which contains the functions defined by Eq. 28.

From a technical point of view, the advantage of the mixed-integer formulation (Eq. 29) is that it allows us to decompose the problem in two separate optimization problems. Using the theoretical framework developed by Geoffrion (Geoffrion and Graves, 1974; Geoffrion, 1972) for the solution of such mixed-integer optimization problems, we can decompose the problem (Eq. 29) into the so-called *primal* and *master* problems.

The primal problem corresponds to problem 29 with fixed $\boldsymbol{w}$-variables (that is, it is in the $\boldsymbol{\tau}$-space only, thus involving only the continuous and real optimization variables). Let $\boldsymbol{w}^k \in \boldsymbol{W}$ be the fixed vector $\boldsymbol{w}$ and N^k be the corresponding fixed number of carbon source switches. Then the primal problem becomes

$$\begin{aligned} &\min_{\boldsymbol{\tau} \in \boldsymbol{T}} f(\boldsymbol{w}^k, \boldsymbol{\tau}) \\ &\text{subject to} \\ &h(\boldsymbol{w}^k, \boldsymbol{\tau}) = 0 \\ &\text{and} \\ &\boldsymbol{g}(\boldsymbol{w}^k, \boldsymbol{\tau}) \leq 0 \end{aligned} \tag{30}$$

Since $2 \leq N^k \leq N_{\max} = 65$, there are exactly 64 such primal problems.

Let us assume that for each one of the 64 primal problems (Eq. 30), there exists a finite solution (the value of the objective function is not $-\infty$). Moreover, let us define by $\upsilon(\boldsymbol{w}^k)$ the finite solution of the primal problem (Eq. 30) for the fixed vector $\boldsymbol{w}^k$. We also define the space: $\boldsymbol{V} = \{\boldsymbol{w}: h(\boldsymbol{w}, \boldsymbol{\tau}) = 0, \boldsymbol{g}(\boldsymbol{w}, \boldsymbol{\tau}) \leq 0 \text{ for some } \boldsymbol{\tau}\}$, which, by construction, consists of those values of $\boldsymbol{w}$, for which the primal problem is feasible (that is, the equality and inequality constraints can be satisfied). $\boldsymbol{W} \cap \boldsymbol{V}$ can be though as the projection of the feasible region of Eq. 29 onto the $\boldsymbol{w}$-space. Then, the master problem is the optimization problem of finding the vector $\boldsymbol{w}$ that minimizes the solutions of all possible primal problems (that is, it is in the $\boldsymbol{w}$-space only, thus involving only the integer optimization variables) and it can be mathematically formulated as follows

$$\begin{aligned} &\min_{\boldsymbol{w}} \upsilon(\boldsymbol{w}) \\ &\textbf{s.t. } \boldsymbol{w} \in \boldsymbol{W} \cap \boldsymbol{V} \end{aligned} \tag{31}$$

The equivalence between the two formulations (Eqs. 29 and 31) is guaranteed by the theoretical result proven by Geoffrion (1970): *If ($\boldsymbol{\tau}^*$, $\boldsymbol{w}^*$) is optimal for Eq. 29, then $\boldsymbol{w}^*$ is optimal for Eq. 31 and vice versa.*

This theoretical result justifies the following approach to the optimization problem under consideration: Find the solutions (global optima) to all 64 primal problems (Eq. 30) (if these solutions exist) and then choose as the optimal solution, the optimum of the 64 optima. We must note that the proposed "brute force" approach is practical in our case due to the relatively small number of possible primal problems. MINLP problems that arise in different applications (Floudas, 1995) are typically large combinatorial problems due to the huge number of 0-1 combinations that can be employed, and can be effectively treated with the use of more sophisticated algorithms such as the Generalized Benders Decomposition (GBD) (Geoffrion, 1972; Floudas et al., 1989; Paules and Floudas, 1989; Manousiouthakis and Sourlas, 1992), Generalized Outer Approximation (GOA) (Duran and Grossmann, 1986a,b). Generalized Cross Decomposition (GCD) (Holmberg, 1992, 1991; Vlachos, 1991) and so on.

Primal Problem

In this section we address the primal problem, which is mathematically expressed by Eq. 30. Since fixing the vector $\boldsymbol{w} \in \boldsymbol{W}$ to a particular set $\boldsymbol{w}^k$, is equivalent to fixing the number N of carbon source switches to a particular value N^k ($2 \leq N^k \leq 65$ and N^k is the number of consecutive 1's in the fixed vector $\boldsymbol{w}^k$), an equivalent formulation of the primal problem is

$$\begin{aligned} &\min_{\boldsymbol{\tau} \in \boldsymbol{T}} f^k(\boldsymbol{\tau}) \\ &\text{subject to} \\ &h^k(\boldsymbol{\tau}) = 0 \\ &\text{and} \\ &\boldsymbol{g}^k(\boldsymbol{\tau}) \leq \boldsymbol{0} \end{aligned} \tag{32}$$

where

$$f^k(\boldsymbol{\tau}) = -\sum_{i=2}^{N^k} C_2^i(\tau_{i-1}, \tau_i) \tag{33}$$

$$h^k(\boldsymbol{\tau}) = (x_{\max} - x_{\min}) \cdot \sum_{i=1}^{N^k} \frac{r_p^i}{E_1^i} \cdot \tau_i - \frac{7}{3}\,\text{CDW} = 0 \tag{34}$$

and $\boldsymbol{g}^k$ is the vector with the following N^k entries

$$g_i^k(\tau_i) = \tau_{\min} - \tau_i \leq 0, \quad i = 1, \ldots, N^k \tag{35}$$

As it will become more clear in the subsequent development, the simplicity (or complexity) of the primal problem analysis depends on the corresponding value of the number of carbon source switches. Based on the value of the quotient: $(x_{\max} - x_{\min}) \cdot \sum_{i=1}^{N^k} (r_p^i / E_1^i)$, we separated the domain of the number of carbon source switches into two regions.

The first corresponds to $2 \leq N^k \leq 6$, for which a simple calculation involving just model parameters, shows that

$$\text{For } 2 \leq N^k \leq 6\text{: } (x_{\max} - x_{\min}) \cdot \sum_{i=1}^{N^k} \frac{r_p^i}{E_1^i} < \frac{7}{3}\,\text{CDW} \tag{36}$$

The second region corresponds to $7 \leq N^k \leq 65$ for which we have

$$\text{For } 7 \leq N^k \leq 65\text{: } (x_{\max} - x_{\min}) \cdot \sum_{i=1}^{N^k} \frac{r_p^i}{E_1^i} > \frac{7}{3}\,\text{CDW} \tag{37}$$

Let us first consider the region of small numbers of carbon source switches: $2 \leq N^k \leq 6$. For this region, the following result holds (see Appendix B for the proof):

Proposition 1. *Consider the optimization problem (Eq. 32). If $2 \leq N^k \leq 6$, then: (a) the global optimum is obtained for $\tau_i \geq 1$, $i = 1, \ldots, N^k$; (b) the global optimum is not strict (unique). More specifically, there exist infinitely many vectors, $\boldsymbol{\tau} \geq 1$, which yield the optimum value of the object function and satisfy the equality and inequality constraints.*

Based on this proposition we see that, for $2 \leq N^k \leq 6$, the global optimum can be explicitly expressed as the sum from 2 up to N^k of the expression given by Eq. A19 in Appendix A.

We will now consider the second region of high numbers of carbon source switches $7 \le N^k \le 65$. For this region, the analysis is more complicated, in the sense that we cannot explicitly express the optimal solution. In this region, the problem expressed by Eq. 32 can be addressed as a classical mathematical programming problem. The necessary optimality conditions for any problem of this type are stated in the following theorem (Bazaraa et al., 1993).

Theorem 1 (Karush-Kuhn-Tucker Necessary Optimality Conditions). *Let **T** be a nonempty open subset of E_n, (the n-dimensional real Euclidean space composed of all real vectors of dimension n) and let f^k, g_i^k, $i = 1, \dots, m$ and h_i^k, $i = 1, \dots, j$ be real valued functions from E_n to E_1. Consider the general problem P:*

$$\begin{aligned} &\text{Minimize} && f^k(\boldsymbol{\tau}) \\ &\text{Subject to} && g_i^k(\boldsymbol{\tau}) \le 0 \quad \text{for } i = 1, \dots, m \\ &&& h_i^k(\boldsymbol{\tau}) = 0 \quad \text{for } i = 1, \dots, j \\ &&& \boldsymbol{\tau} \in \boldsymbol{T} \end{aligned}$$

Let $\boldsymbol{\tau}^*$ be a feasible solution (that is, $\boldsymbol{\tau}^* \in \boldsymbol{\Omega} = \{\boldsymbol{\tau}: h_i^k(\boldsymbol{\tau}) = 0$ for $i = 1, \dots, j$ and $g_i^k(\boldsymbol{\tau}) \le 0$ for $i = 1, \dots, m\}$), and let $I = \{i: g_i(\boldsymbol{\tau}) = 0\}$. Suppose that f^k and g_i^k for $i = 1, \dots, m$ are differentiable at $\boldsymbol{\tau}^*$, and that each h_i^k for $i = 1, \dots, j$ is continuously differentiable at $\boldsymbol{\tau}^*$. Further, suppose that $\nabla g_i^k(\boldsymbol{\tau}^*)$ for $i \in I$ and $\nabla h_i^k(\boldsymbol{\tau}^*)$ for $i = 1, \dots, j$ are linearly independent. If $\boldsymbol{\tau}^*$ solves problem P locally, then there exist unique scalars λ_i^*, $i = 1, \dots, j$ and μ_i^*, $i = 1, \dots, m$, such that the following conditions hold.

$$\nabla f^k(\boldsymbol{\tau}^*) + \sum_{i=1}^{j} \lambda_i^* \nabla h_i^k(\boldsymbol{\tau}^*) + \sum_{i=1}^{m} \mu_i^* \nabla g_i^k(\boldsymbol{\tau}^*) = 0 \quad (38)$$

$$\mu_i^* g_i^k(\boldsymbol{\tau}^*) = 0 \qquad i = 1, \dots, m \quad (39)$$

$$\mu_i^* = \nu_i^2 \ge 0 \qquad i = 1, \dots, m \quad (40)$$

Conditions 39 are called the Karush-Kuhn-Tucker (KKT) complementarity conditions, whereas conditions 38 are called the KKT gradient conditions. These gradient conditions can be represented more compactly as

$$\nabla L^k(\boldsymbol{\tau}^*) = 0 \quad (41)$$

where the scalar function L is the restricted Lagrangian defined as

$$L^k(\boldsymbol{\tau}) \equiv \phi^k(\boldsymbol{\tau}, \boldsymbol{\lambda}^*, \boldsymbol{\mu}^*) = f^k(\boldsymbol{\tau}) + \sum_{i=1}^{j} \lambda_i^* h_i^k(\boldsymbol{\tau}) + \sum_{i=1}^{m} \mu_i^* g_i^k(\boldsymbol{\tau}) \quad (42)$$

The augmented Lagrangian ϕ is not restricted to the particular values of the Lagrange multipliers that satisfy conditions 38–40 and is defined as

$$\phi^k(\boldsymbol{\tau}, \boldsymbol{\lambda}, \boldsymbol{\mu}) = f^k(\boldsymbol{\tau}) + \sum_{i=1}^{k} \lambda_i h_i^k(\boldsymbol{\tau}) + \sum_{i=1}^{m} \mu_i g_i^k(\boldsymbol{\tau}) \quad (43)$$

In order to sustain consistency in the notation, we used the same notation for the objective and constraint defining functions as before (see expressions 33–35). More specifically, the superscript k in the state of the theorem, as well as in expressions 38–43 is used to denote the forms of the objective, constraint defining, restricted and augmented Lagrangian functions that correspond to the primal problem with N^k carbon source switches.

For our particular optimization problem, the set $\boldsymbol{T}$ (defined in the previous section as the set of all N-dimensional vectors with positive entries) is obviously an open nonempty subset of E_n. Also, there is only one equality constraint ($j = 1$) and N^k inequality constraints ($m = N^k$). Furthermore, the objective function (Eq. 33), the equality constraint (Eq. 34), and all inequality constraints (Eq. 35) are continuously differentiable $\forall \boldsymbol{\tau} \in \boldsymbol{T}$. Moreover, since we only have one equality constraint, its gradient is linearly independent. Also, from Eq. 35, we can easily see that the gradients $\nabla g_i^k(\boldsymbol{\tau})$ are N^k dimensional vectors with all entries equal to zero except the ith entry which is always equal to -1. Thus, the gradients $\nabla g_i^k(\boldsymbol{\tau})$ are linearly independent $\forall \boldsymbol{\tau}$ and $\forall i \in I$ (that is, the set of active inequality constraints). Therefore, according to theorem 1, if the optimization problem (Eq. 32) has a finite local solution, then conditions 38–40 must be satisfied.

The following proposition limits any local solution of problem 32 (if it exists) to the set: $\boldsymbol{S} = \{\boldsymbol{\tau}: h(\boldsymbol{\tau}) = 0,\ \boldsymbol{g}(\boldsymbol{\tau}) \le \boldsymbol{0}$, and $\boldsymbol{\tau} < \boldsymbol{1}\}$. The proof is given in Appendix B.

Proposition 2. *If inequality 37 holds (that is, for $7 \le N^k \le 65$), and if there exists a local solution to the primal problem (Eq. 32), then it must satisfy: $\boldsymbol{\tau} \in \boldsymbol{S} = \{\boldsymbol{\tau} \in \boldsymbol{T}: h(\boldsymbol{\tau}) = 0,\ \boldsymbol{g}(\boldsymbol{\tau}) \le 0$, and $\boldsymbol{\tau} < \boldsymbol{1}\}$.*

Since, for $7 \le N^k \le 65$, proposition 2 excludes the possibility of any of the dimensionless switching times that satisfy the KKT gradient, complementarity and nonnegativity conditions, to be greater or equal to 1, we can reformulate the primal problem (Eq. 32) as follows

$$\begin{aligned} &\text{minimize } \left\{ f^k(\boldsymbol{\tau}) : \boldsymbol{\tau} \in \boldsymbol{S} \right\} \\ &\text{where } \boldsymbol{S} = \left\{ \boldsymbol{\tau} \in \boldsymbol{T} : h(\boldsymbol{\tau}) = 0, \boldsymbol{g}(\tau) \le \boldsymbol{0}, \text{and } \boldsymbol{\tau} < \boldsymbol{1} \right\} \end{aligned} \quad (44)$$

The solution of the system of the KKT necessary optimality conditions (Eq. 38–40) will provide (if it exists) a candidate for the optimum. Lemma 1 that follows (Bazaraa et al., 1993) provides sufficient optimality conditions and will allow us to establish that the solution is indeed the optimal.

Lemma 1 (Sufficient Optimality Conditions). *Consider the optimization problem defined in Eq. 44, where the objective and constraint defining functions, are all twice differentiable, and where **T** is a nonempty subset of E_n. Suppose that $\boldsymbol{\tau}^*$ is a KKT point (that is, satisfies conditions 38–40) with Lagrange multipliers $\boldsymbol{\mu}^*$ and λ^* associated with the inequality and equality constraints, respectively. Define the restricted Lagrangian function $L^k(\boldsymbol{\tau})$ as in Eq. 42 and denote its Hessian as $\nabla^2 L^k(\boldsymbol{\tau})$. If $\nabla^2 L^k(\boldsymbol{\tau})$ is positive definite $\forall \boldsymbol{\tau} \in \boldsymbol{S}$, then $\boldsymbol{\tau}^*$ is the strict (unique) global optimum solution of problem (44), and hence problem 32.*

For the optimization problem 44, $\boldsymbol{T}$ is a nonempty subset of E_n, while the objective function (Eq. 33) is twice differentiable as the sum of N^k twice differentiable functions (see expressions in Appendix A). Moreover, the equality and inequality constraints are linear and, as such, are twice differentiable as well. Due to the linearity of all constraints, it is easy to see that the Hessian of the restricted Lagrangian is identical to the Hessian of the objective function [that is, $\nabla^2 L^k(\boldsymbol{\tau}) = \nabla^2 f^k(\boldsymbol{\tau})$].

Let us now define the set $\boldsymbol{S}' = \{\boldsymbol{\tau} \in \boldsymbol{T}: \boldsymbol{g}(\boldsymbol{\tau}) \leq \boldsymbol{0}$ and $\boldsymbol{\tau} < \boldsymbol{1}\}$. Clearly: $\boldsymbol{S} \subset \boldsymbol{S}'$. For this set $\boldsymbol{S}'$, the following lemma holds (see Appendix B for the proof):

Lemma 2. *The Hessian of the objective function 33 ($\nabla^2 f^k(\boldsymbol{\tau})$) is positive definite* $\forall \boldsymbol{\tau} \in \boldsymbol{S}' = \{\boldsymbol{\tau} \in \boldsymbol{T}: \boldsymbol{g}(\boldsymbol{\tau}) \leq \boldsymbol{0}$ *and* $\boldsymbol{\tau} < \boldsymbol{1}\}$.

Since $\boldsymbol{S}$ is a subset of $\boldsymbol{S}'$, Lemma 2 guarantees that the Hessian of the objective function is positive definite in $\boldsymbol{S}$ as well. As explained above, this implies that the Hessian of the restricted Lagrangian is also positive definite in $\boldsymbol{S}$. According to the definition of a strictly convex function (see Canon et al., 1970 for a definition), the strict positive definiteness of the Hessian implies that the objective function is strictly convex. Thus, all conditions of Lemma 1 are satisfied. Therefore, if a KKT point exists, it is unique and yields the globally optimum solution of the problem.

Summarizing the results of this section, we can state the following:

(a) For $2 \leq N^k \leq 6$ the global optimum is just the sum from 2 up to N^k of the expression given by Eq. A19 in Appendix A. This global optimum is not unique. It can be obtained for an infinite number of dimensionless switching time sets that satisfy the equality constraint and are such that: $\boldsymbol{\tau} \geq \boldsymbol{1}$.

(b) For $7 \leq N^k \leq 65$, if a KKT point $\boldsymbol{\tau}^*$ and corresponding Lagrange multipliers $\boldsymbol{\mu}^*$ and λ^* exist (that is, satisfying conditions 38–40 the equality constraint 34 and the inequality constraints 35, then (1) this point is unique, (2) it is such that $\boldsymbol{\tau}^* < \boldsymbol{1}$, and (3) yields the global optimum solution of the primal problem 33.

Results

As it is apparent from the conclusions of the previous section, the difference between the two regions is that for $2 \leq N^k \leq 6$, the global optimum is not unique, whereas for $7 \leq N^k \leq 65$ it is. Moreover, for $2 \leq N^k \leq 6$, the global optimum can be explicitly computed by using Eq. A19.

On the contrary, for $7 \leq N^k \leq 65$, the unique solution to the primal problem 32, should satisfy Eqs. 38–40 as well as the equality constraint (Eq. 34) and inequality constraints (Eq. 35). Due to the highly nonlinear nature of the objective function (see equations in Appendix A), an analytical solution cannot be obtained. For this purpose, the problem was numerically addressed with the use of the Newton-Raphson algorithm with a variable step (see Avriel, 1976). The value of this variable step at each Newton-Raphson iteration was obtained by performing a unidimensional search to find the value that minimizes the L_2 norm of the residual vector. In each case $7 \leq N^k \leq 65$ convergence was achieved. It was found that for $7 \leq N^k \leq 35$ none of the inequality constraints was active (that is, $g_i \leq 0$, $\forall i = 1, \ldots, N^k$), whereas for $36 \leq N^k \leq 65$ some inequality constraints were active.

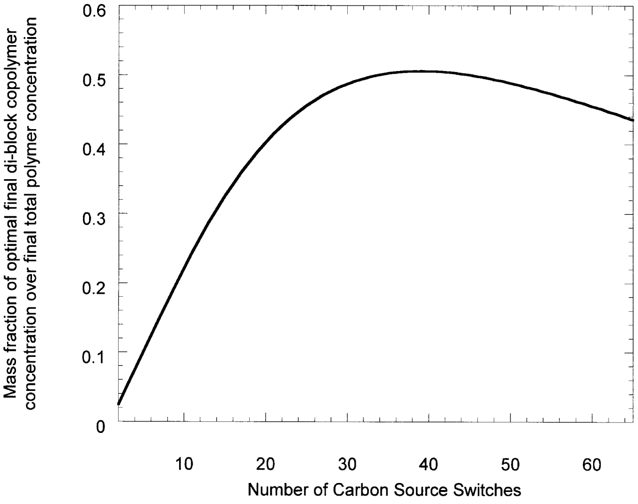

Figure 1. Optimal solution of all possible primal problems ($2 \leq N \leq 65$) as a function of the number of carbon source switches.

Mass fraction of the optimal final diblock copolymer concentration over the final total polymer concentration (7/3 CDW).

Figure 1 shows the mass fraction of the optimal final diblock copolymer concentration produced [$-f^k(\boldsymbol{\tau})$] over the total polymer concentration produced after N^k carbon source switches for $2 \leq N^k \leq 65$ (entire region of number of carbon source switches). Since the equality constraint (Eq. 34) is always satisfied, this total polymer concentration is always equal to 7/3 CDW. Thus, the functional dependence of the actual optimal final concentrations on the number of carbon source switches will be identical to the one depicted in the figure for the final fractions.

Notice that the mass fraction of diblock copolymers reaches a maximum value of 50.6% at $N = 39$ switches. Table 2 shows the corresponding optimal dimensionless and dimensional switching times. According to the analysis presented earlier, this is the global optimum of the original optimization problem 29. One can physically understand the existence of a maximum as follows: As the number of switches increases, the number of diblock copolymer producing stages increases. On the other hand, the switching times decrease causing a decrease in the amount of diblock copolymers that terminates at each stage, as well as the amount of active monoblock copolymers that will produce diblock copolymers at the next stage. The interaction of these two competing effects of the increase in the number of stages is responsible for the observed maximum. We note the following interesting features:

(a) The global optimum is obtained for the same value of carbon source switches as in the case where the assumption of equal dimensionless switching times was made (Mantzaris et al., 2001b).

(b) The value of the global optimum is only slightly higher than the value that was obtained for $N = 39$ carbon source switches under the assumption of equal dimensionless switching times (50.6% vs. 50.05%) (Mantzaris et al., 2001b).

(c) As it is shown in Table 2, at the global optimum, the inequality constraints are active only for the first and last stages of the process. Moreover, the optimal dimensionless switching times are approximately symmetric around the middle (20th) stage of the process: Around this middle stage, the dimensional switching times remain approximately the same

Table 2. Optimal Solution

Stage	Dimensionless Switching Time	Dimensional (min) Switching Time
1 (PHBV)	0.10000	16.977
2 (PHB)	0.14936	54.457
3 (PHBV)	0.17640	29.947
4 (PHB)	0.16686	60.837
5 (PHBV)	0.17934	30.446
6 (PHB)	0.16729	60.996
7 (PHBV)	0.17941	30.457
8 (PHB)	0.16730	60.999
9 (PHBV)	0.17941	30.457
10 (PHB)	0.16730	60.999
11 (PHBV)	0.17941	30.457
12 (PHB)	0.16730	60.999
13 (PHBV)	0.17941	30.457
14 (PHB)	0.16730	60.999
15 (PHBV)	0.17941	30.457
16 (PHB)	0.16730	60.999
17 (PHBV)	0.17941	30.457
18 (PHB)	0.16730	60.999
19 (PHBV)	0.17941	30.457
20 (PHB)	0.16730	60.999
21 (PHBV)	0.17941	30.457
22 (PHB)	0.16730	60.999
23 (PHBV)	0.17941	30.457
24 (PHB)	0.16730	60.999
25 (PHBV)	0.17941	30.457
26 (PHB)	0.16730	60.999
27 (PHBV)	0.17941	30.457
28 (PHB)	0.16730	60.999
29 (PHBV)	0.17941	30.457
30 (PHB)	0.16730	60.999
31 (PHBV)	0.17941	30.457
32 (PHB)	0.16730	60.999
33 (PHBV)	0.17941	30.457
34 (PHB)	0.16729	60.996
35 (PHBV)	0.17934	30.446
36 (PHB)	0.16687	60.841
37 (PHBV)	0.17640	29.946
38 (PHB)	0.14971	54.583
39 (PHBV)	0.10000	16.977

and equal to 30.5 min for each PHBV producing stage and 61 min for each PHB producing stage. As we get closer to the first or last stages of the process, the dimensionless switching times become gradually smaller. This shows that the ad hoc assumption of equal dimensionless switching times (corresponding to a relationship of PHBV dimensional switching time/PHB dimensional switching time = 1:2) that was made in our previous work (Mantzaris et al., 2001b) turned out to be very close to the optimum.

Figure 2 shows the mass fractions of block copolymer, diblock copolymer, and triblock copolymer concentrations over the total polymer concentration produced, as a function of time, for $N = 39$ carbon source switches and for the dimensionless switching times presented in Table 2, where the global maximum for the final diblock copolymer concentration is observed. The final mass fraction of the total block copolymer is approximately 68.8%, whereas the final mass fraction of triblock copolymer is approximately 13%. Thus, at the optimum number of switches, 50.6% of the total polymer produced is diblock copolymer. This represents 73.5% of the total block copolymer produced. Moreover, the final concentration of triblock copolymers is 13% of the total polymer produced and 18.9% of the total block copolymer produced. Therefore, as the optimal number of carbon source switches, the block copolymers, which contain more than three blocks represent only 5.2% of the total polymer produced and 7.6% of the total block copolymer produced.

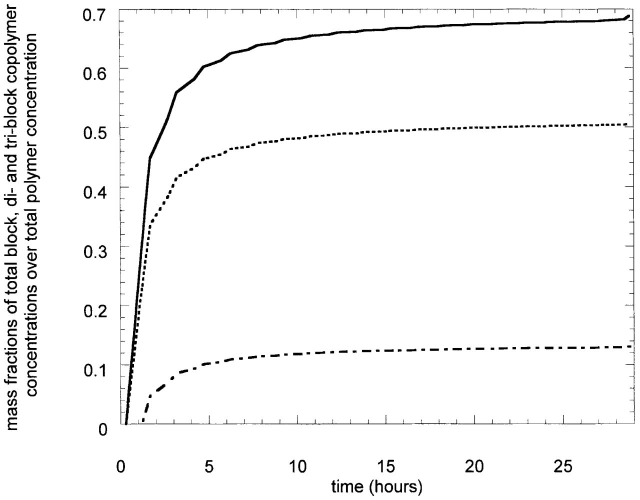

Figure 2. Time evolution of mass fractions of total block copolymer, di- and triblock copolymer concentration for the optimal number of carbon source switches ($N = 39$).

Solid line, total block copolymer; dotted line, diblock copolymer; dashed-dotted line, triblock copolymer.

Summary and Conclusions

In this work, we addressed the problem of finding the optimal number of carbon source switches and corresponding switching times that maximize the final concentration of diblock copolymers (PHB-PHBV and PHBV-PHB). The end of the process was implicitly defined as the time when the concentration of the total polymer produced is 70% of the total cell dry weight. Moreover, we assumed that no stage of the process where less than 50 g/L of total polymer is produced can exist.

The problem under consideration was mathematically formulated in the mixed-integer nonlinear programming (MINLP) framework, which allowed the decomposition of the original problem into the primal and master problems. The primal problem corresponds to finding the optimal set of switching times for a fixed number of carbon source switches, whereas the master problem consists of finding the optimal number of carbon source switches that optimizes the optimal solutions of all possible primal problems. The solution to each possible primal problem was obtained numerically with the use of the Newton-Raphson algorithm with a variable step. It was theoretically shown that each solution thus obtained is the unique global optimum for each primal problem in the region $7 \leq N \leq 65$. On the other hand, for $2 \leq N \leq 6$, it was established that the analytically evaluated global optimum of each primal problem, is not unique.

The global optimum of the original problem was obtained for 39 carbon source switches. It corresponds to a mass fraction of 50.6% of final diblock copolymer concentration of final total polymer concentration. The result is very close to the one obtained in our previous work (Mantzaris et al., 1999b) under the ad hoc assumption of equal dimensionless switching times. Furthermore, the optimal dimensionless switching times were approximately symmetric around the middle (20th) stage of the process: Around this middle stage, the dimensional switching times remain approximately the same and equal to 30.5 min for each PHBV producing stage and 61 min for each PHB producing stage. As we get closer to the first or last stages of the process, the dimensionless switching times become gradually smaller.

This work not only answers the complicated problem under consideration, but also serves as an illustrative example, for the general methodology that can rigorously address similar optimization problems, such as maximizing the final triblock copolymer concentration, for example. The corresponding problem although very similar to the one presented here, is more complicated, because the concentration of triblock copolymers produced at each stage of the process depends on three switching times, as opposed to just two in the case of diblock copolymers. Finally, the type of information that was gained with the simultaneous use of the proposed mathematical model and the presented analysis of the optimization problem cannot be obtained by experiment. The presented approach is therefore very useful for obtaining system design parameters that can enable the formation of specific block-copolymers of interest.

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Appendix A

In this appendix we present the analytical expressions of the concentration of diblock copolymers produced at the ith stage of the carbon source switching process and the corresponding first and second partial derivatives with respect to the dimensionless switching times that they depend on (τ_i, τ_{i-1}), as explicit functions of τ_i, τ_{i-1}. In order to simplify the expressions, we first define the following parameters

$$k = (x_{\max} - x_{\min}) \tag{A1}$$

$$y_1^i = -\frac{1-\sqrt{1-4t_2^it_3^i}}{2t_3^i} \tag{A2}$$

$$y_2^i = -\frac{1+\sqrt{1-4t_2^it_3^i}}{2t_3^i} \tag{A3}$$

$$a^i = \frac{-t_1^i}{2t_3^i} + \frac{t_1^i}{2t_3^i\sqrt{1-4t_2^it_3^i}} \tag{A4}$$

$$b^i = \frac{-t_1^i}{2t_3^i} - \frac{t_1^i}{2t_3^i\sqrt{1-4t_2^it_3^i}} \tag{A5}$$

$$D^i = \frac{N_A r_i^i}{E_1^i x_{\min}} \left(\frac{1-\sqrt{1-4t_2^it_3^i}}{2t_3^i}\right)^{\frac{t_1^i}{2t_3^i} - \frac{t_1^i}{2t_3^i\sqrt{1-4t_2^it_3^i}}} \cdot \left(\frac{1+\sqrt{1-4t_2^it_3^i}}{2t_3^i}\right)^{\frac{t_1^i}{2t_3^i} + \frac{t_1^i}{2t_3^i\sqrt{1-4t_2^it_3^i}}} \tag{A6}$$

We also define the following functions of the dimensionless molecular weight y $(0 \le y \le 1)$

$$r_t^i(y) = \frac{t_1^i y}{t_2^i + y + t_3^i y^2} \tag{A7}$$

$$f_1(y) = (ky + x_{\min}) \cdot r_t^i(y) \cdot \left(y - y_1^i\right)^{a^i} \cdot \left(y - y_2^i\right)^{b^i} \tag{A8}$$

$$f_2(y, \tau') = \left(y - y_1^{i-1} - \tau'\right)^{a^{i-1}} \cdot \left(y - y_2^{i-1} - \tau'\right)^{b^{i-1}} \cdot \left(y - y_1^i - \tau'\right)^{-a^i} \cdot \left(y - y_2^i - \tau'\right)^{-b^i} \tag{A9}$$

From Eq. A9 we also obtain

$$\frac{\partial f_2(y,\tau')}{\partial \tau'} = \left(- \frac{a^{i-1}}{\left(y - y_1^{i-1} - \tau'\right)} - \frac{b^{i-1}}{\left(y - y_2^{i-1} - \tau'\right)} + \frac{a^i}{\left(y - y_1^i - \tau'\right)} + \frac{b^i}{\left(y - y_2^i - \tau'\right)} \right) \cdot f_2(y, \tau') \tag{A10}$$

The superscripts in Eqs. A1–A10, and in the following, correspond to the stage of the process. The expressions depend on both the relative and absolute magnitude of τ_i, τ_{i-1}. We define also the dimensionless times

$$\begin{aligned} \tau_d &= \min\{\tau_{i-1}, \tau_i\} \\ \tau_u &= \max\{\tau_{i-1}, \tau_i\} \\ \tau_c &= \min\{1, \tau_{i-1} + \tau_i\} \end{aligned} \tag{A11}$$

We distinguish between three cases

Case I. $\tau_d \le \tau_u < 1$.

If $\tau_d = \tau_{i-1}$ then

$$C_2^i(\tau_{i-1}, \tau_i) = \frac{kD^{i-1}}{N_A} \left\{ \int_0^{\tau_{i-1}} f_1(y) \left[\int_0^y f_2(y,\tau') d\tau' \right] dy + \int_{\tau_{i-1}}^{\tau_i} f_1(y) \left[\int_{y-\tau_{i-1}}^{y} f_2(y,\tau') d\tau' \right] dy + \int_{\tau_i}^{\tau_c} f_1(y) \times \left[\int_{y-\tau_{i-1}}^{\tau_i} f_2(y,\tau') d\tau' \right] dy \right\} \tag{A12a}$$

whereas if $\tau_d = \tau_i$ then

$$C_2^i(\tau_{i-1}, \tau_i) = \frac{kD^{i-1}}{N_A} \left\{ \int_0^{\tau_i} f_1(y) \left[\int_0^y f_2(y,\tau') d\tau' \right] dy + \int_{\tau_i}^{\tau_{i-1}} f_1(y) \left[\int_0^{\tau_i} f_2(y,\tau') d\tau' \right] dy + \int_{\tau_{i-1}}^{\tau_c} f_1(y) \times \left[\int_{y-\tau_{i-1}}^{\tau_i} f_2(y,\tau') d\tau' \right] dy \right\} \tag{A12b}$$

The expressions of the first and second derivatives do not depend on the relative magnitude of τ_i, τ_{i-1}. The first derivatives are expressed as follows

$$\frac{\partial C_2^i(\tau_{i-1}, \tau_i)}{\partial \tau_{i-1}} = \frac{kD^{i-1}}{N_A} \left[\int_{\tau_{i-1}}^{\tau_c} f_1(y) \cdot f_2(y, y - \tau_{i-1}) dy \right] \tag{A13}$$

$$\frac{\partial C_2^i(\tau_{i-1}, \tau_i)}{\partial \tau_i} = \frac{kD^{i-1}}{N_A} f_2(\tau_i + \tau_{i-1}, \tau_i) \cdot \int_{\tau_i}^{\tau_c} f_1(y) dy \tag{A14}$$

The two second mixed derivatives are equal to each other and are expressed as follows

$$\frac{\partial^2 C_2^i(\tau_{i-1}, \tau_i)}{\partial \tau_{i-1} \partial \tau_i} = \frac{\partial^2 C_2^i(\tau_{i-1}, \tau_i)}{\partial \tau_i \partial \tau_{i-1}} = \begin{cases} \dfrac{kD^{i-1}}{N_A} f_1(\tau_i + \tau_{i-1}) \cdot f_2(\tau_i + \tau_{i-1}, \tau_i), & \tau_c = \tau_i + \tau_{i-1} \\ 0, & \tau_c = 1 \end{cases} \tag{A15}$$

while the two second derivatives are given by

$$\frac{\partial^2 C_2^i(\tau_{i-1}, \tau_i)}{\partial \tau_i^2} = \frac{\partial^2 C_2^i(\tau_{i-1}, \tau_i)}{\partial \tau_i \partial \tau_{i-1}} - \frac{kD^{i-1}}{N_A} \times \left[f_1(\tau_i) \cdot f_2(\tau_i, \tau_i) - \int_{\tau_i}^{\tau_c} f_1(y) \cdot \frac{\partial f_2(y, \tau_i)}{\partial \tau'} dy \right] \tag{A16}$$

$$\frac{\partial^2 C_2^i(\tau_{i-1}, \tau_i)}{\partial \tau_{i-1}^2} = \frac{\partial^2 C_2^i(\tau_{i-1}, \tau_i)}{\partial \tau_i \partial \tau_{i-1}} - \frac{kD^{i-1}}{N_A} \times \left[f_1(\tau_{i-1}) \cdot f_2(\tau_i + \tau_{i-1}, \tau_i) - \frac{\partial f_2(\tau_i + \tau_{i-1}, \tau_i)}{\partial \tau'} \cdot \int_{\tau_{i-1}}^{\tau_c} f_1(y) dy \right] \tag{A17}$$

Case II. $\tau_d < 1 \le \tau_u$.

If $\tau_d = \tau_{i-1}$, then

$$C_2^i(\tau_{i-1}, \tau_i) = C_2^i(\tau_{i-1}) = \frac{kD^{i-1}}{N_A} \left\{ \int_0^{\tau_{i-1}} f_1(y) \left[\int_0^y f_2(y,\tau') d\tau' \right] dy + \int_{\tau_{i-1}}^{1} f_1(y) \left[\int_{y-\tau_{i-1}}^{y} f_2(y,\tau') d\tau' \right] dy \right\} \tag{A18a}$$

whereas if $\tau_d = \tau_i$ then

$$C_2^i(\tau_{i-1}, \tau_i) = C_2^i(\tau_i) = \frac{kD^{i-1}}{N_A} \left\{ \int_0^{\tau_{i-1}} f_1(y) \left[\int_0^y f_2(y,\tau') d\tau' \right] dy + \int_{\tau_i}^{1} f_1(y) \left[\int_0^{\tau_i} f_2(y,\tau') d\tau' \right] dy \right\} \tag{A18b}$$

Notice that in this case, $C_2^i(\tau_{i-1}, \tau_i)$ is independent of the τ_u. Thus, the first and second derivatives with respect to this dimensionless switching time, are zero. The corresponding expressions for the first and second derivatives w.r.t. τ_d are given by the same expressions as above with $\tau_c = 1$.

Case III. $1 \le \tau_d \le \tau_u$.

$$C_2^i(\tau_{i-1},\tau_i) = C_2^i = \frac{kD^{i-1}}{N_A}\left\{\int_0^1 f_1(y)\left[\int_0^y f_2(y,\tau')d\tau'\right]dy\right\} \tag{A19}$$

Notice that in this case, $C_2^i(\tau_{i-1}, \tau_i)$ is a constant independent of both τ_i and τ_{i-1}. Thus, all first and second derivatives are zero.

By a simple inspection of the derived expressions, one can make the following remarks:

(a) Since the integrands in both first derivative formulae are always non-negative, $C_2^i(\tau_{i-1}, \tau_i)$ is a monotonically increasing function of both dimensionless switching times. Each of the first derivatives becomes zero when the corresponding dimensionless switching time becomes equal to 1.

(b) If one of the two dimensionless switching times is greater or equal to 1, then $C_2^i(\tau_{i-1}, \tau_i)$ is independent of this switching time. Thus, based also on remark (a), $C_2^i(\tau_{i-1}, \tau_i)$ obtains its maximum value for $\tau_{i-1} = 1$ and $\tau_i = 1$. For larger values of τ_i, τ_{i-1}, C_2^i, $C_2^i(\tau_{i-1}, \tau_i)$ remains constant and equal to its maximum value.

(c) The mixed second derivative

$$\frac{\partial^2 C_2^i(\tau_{i-1}, \tau_i)}{\partial\tau_{i-1}\partial\tau_i}$$

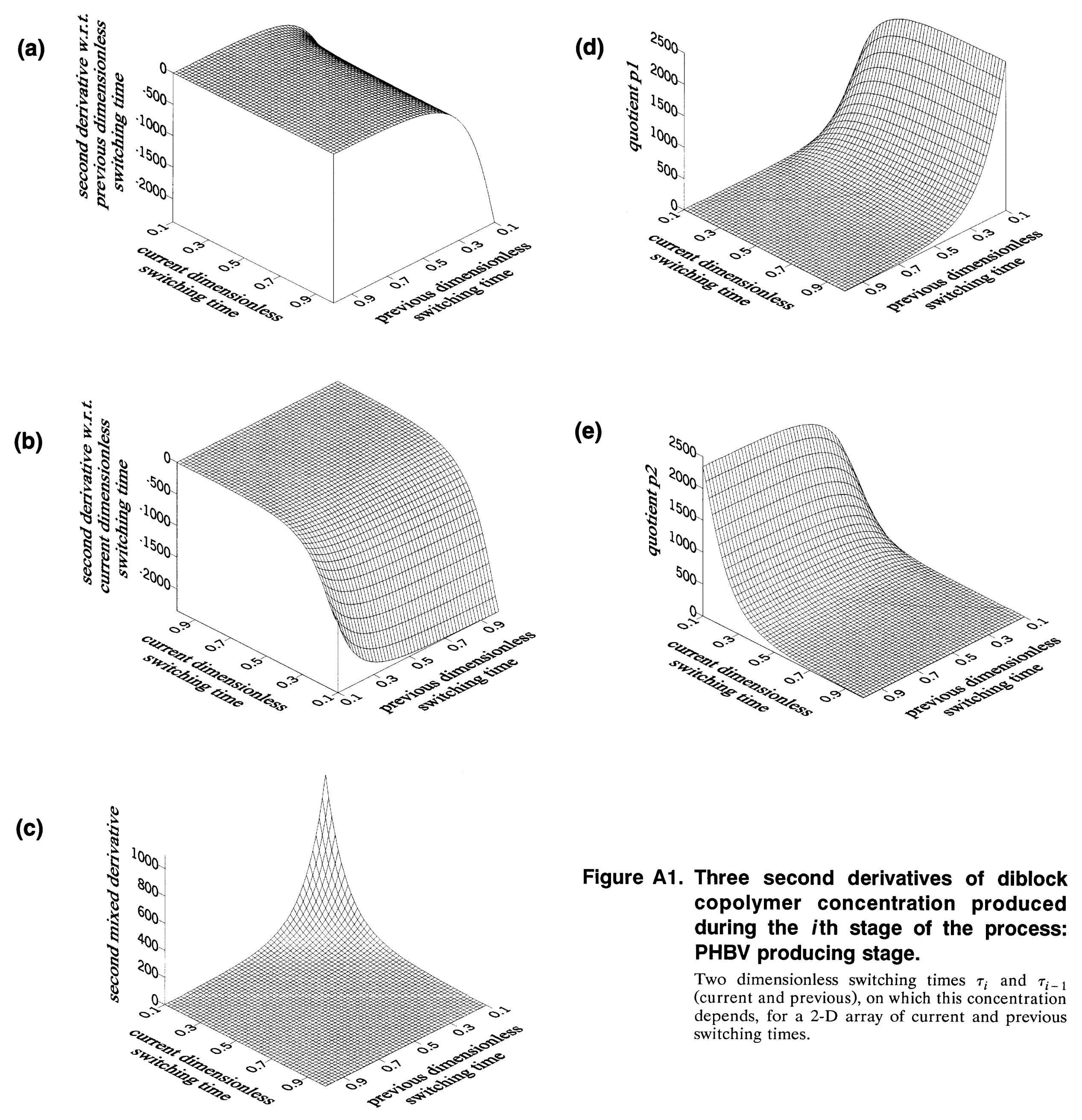

Figure A1. Three second derivatives of diblock copolymer concentration produced during the *i*th stage of the process: PHBV producing stage.

Two dimensionless switching times τ_i and τ_{i-1} (current and previous), on which this concentration depends, for a 2-D array of current and previous switching times.

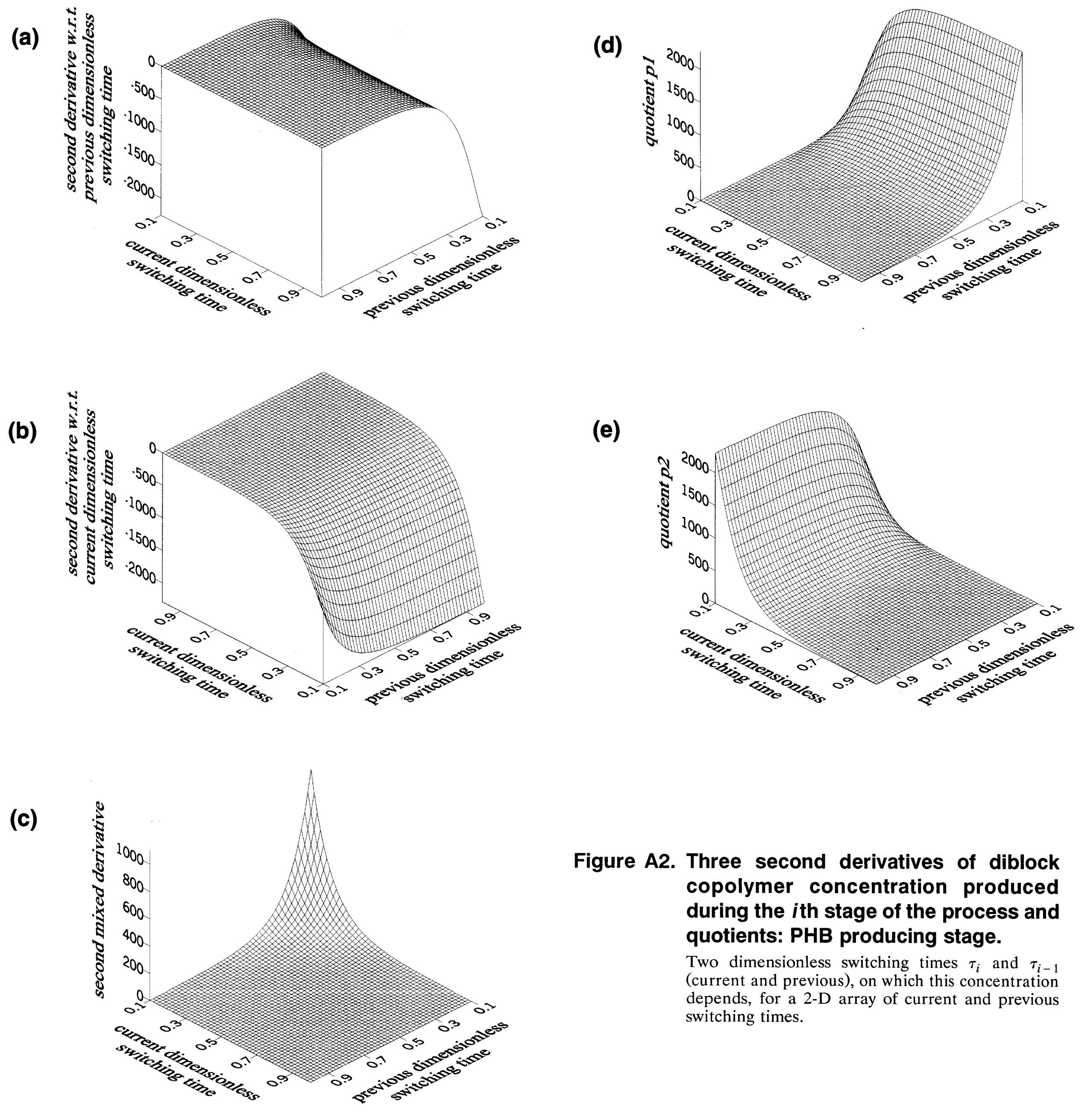

Figure A2. Three second derivatives of diblock copolymer concentration produced during the *i*th stage of the process and quotients: PHB producing stage.

Two dimensionless switching times τ_i and τ_{i-1} (current and previous), on which this concentration depends, for a 2-D array of current and previous switching times.

is always positive for $\tau_i + \tau_{i-1} < 1$ and zero for $\tau_i + \tau_{i-1} \geq 1$.

The expressions of the second derivatives:

$$\frac{\partial^2 C_2^i(\tau_{i-1}, \tau_i)}{\partial \tau_{i-1}^2}, \frac{\partial^2 C_2^i(\tau_{i-1}, \tau_i)}{\partial \tau_i^2}$$

for dimensionless switching times, strictly less than 1, are even more complicated. In order to examine their behavior, we computed them for an array of dimensionless switching times: τ_i, $\tau_{i-1} \in [0.1, 1]$. The results are shown in Figures A1 and A2 for the cases of PHBV and PHB formation, respectively. Moreover, we also show the second mixed derivatives and the values of the quotients:

$$p_1 = \left| \frac{\partial^2 C_2^i(\tau_{i-1}, \tau_i)}{\partial \tau_{i-1}^2} \right| - \left| \frac{\partial^2 C_2^i(\tau_{i-1}, \tau_i)}{\partial \tau_{i-1} \partial \tau_i} \right|$$

$$\text{and } p_2 = \left| \frac{\partial^2 C_2^i(\tau_{i-1}, \tau_i)}{\partial \tau_i^2} \right| - \left| \frac{\partial^2 C_2^i(\tau_{i-1}, \tau_i)}{\partial \tau_{i-1} \partial \tau_i} \right|.$$

Based on the results shown in these figures, we can make the following remarks:

(d) Both

$$\frac{\partial^2 C_2^i(\tau_{i-1},\tau_i)}{\partial \tau_{i-1}^2} \quad \text{and} \quad \frac{\partial^2 C_2^i(\tau_{i-1},\tau_i)}{\partial \tau_i^2}$$

are nonpositive for each pair τ_i, $\tau_{i-1} \in [0.1, 1]$.

$$\frac{\partial^2 C_2^i(\tau_{i-1},\tau_i)}{\partial \tau_{i-1}^2} \left(\frac{\partial^2 C_2^i(\tau_{i-1},\tau_i)}{\partial \tau_i^2} \right)$$

becomes zero for $\tau_{i-1} = 1$ ($\tau_i = 1$). Thus, for each pair τ_i, $\tau_{i-1} \in [0.1, 1)$, both derivatives are strictly negative.

(e) $$\frac{\partial^2 C_2^i(\tau_{i-1},\tau_i)}{\partial \tau_{i-1}^2}$$

is an increasing function of the τ_{i-1} and a decreasing function of τ_i.

(f) $$\frac{\partial^2 C_2^i(\tau_{i-1},\tau_i)}{\partial \tau_i^2}$$

is a decreasing function of τ_{i-1} and an increasing function of τ_i.

(g) The mixed second derivative $\partial^2 C_2^i(\tau_{i-1},\tau_\mathrm{i})/\partial\tau_{i-1}\partial\tau_i$ is a decreasing function of both τ_i, $\tau_{i-1} \in [0.1, 1]$. Thus, it obtains its maximum value for the minimum possible values for the two dimensionless switching times.

(h) Since p_1, $p_2 > 0$, for each pair $\tau_i, \tau_{i-1} \in [0.1, 1)$, each of the two second derivatives

$$\frac{\partial^2 C_2^i(\tau_{i-1},\tau_i)}{\partial \tau_{i-1}^2} \text{ and } \frac{\partial^2 C_2^i(\tau_{i-1},\tau_i)}{\partial \tau_i^2}$$

is strictly greater in magnitude than the corresponding second mixed derivative

$$\frac{\partial^2 C_2^i(\tau_{i-1},\tau_i)}{\partial \tau_{i-1},\partial \tau_i},$$

for each pair τ_i, $\tau_{i-1} \in [0.1, 1)$.

Appendix B

In this appendix we prove propositions 1, 2 and lemma 2 which were presented in the text and were used in the analysis of the primal problem.

Proposition 1. *Consider the optimization problem (Eq. 32). If $2 \le N^k < 6$, then: (a) the global optimum is obtained for $\tau_i \ge 1$, $i = 1, \ldots, N^k$; (b) the global optimum is not strict (unique). More specifically, there exist infinitely many vectors $\boldsymbol{\tau} \ge \mathbf{1}$, which yield the optimum value of the objective function and satisfy the equality and inequality constraints.*

Proof: Since $2 \le N^k \le 6$, inequality 36 holds. Inequality 36 implies that in order to satisfy the equality constraint 34, there must exist at least one dimensionless switching time $\tau_i \ge 1$, *for some* $i \in [1, N^k]$. Thus, it is also possible to have: $\tau_i \ge 1$ *for all* $i \in [1, N^k]$ (or equivalently: $\boldsymbol{\tau} \ge \mathbf{1}$) and still satisfy Eq. 34.

As was shown in Appendix A, $C_2^i(\tau_{i-1}, \tau_i)$ is a monotonically increasing function of both τ_{i-1}, τ_i and it obtains its maximum value for $\tau_{i-1} = 1$ and $\tau_i = 1$. For larger values of τ_i, τ_{i-1}, $C_2^i(\tau_{i-1}, \tau_i)$ remains constant and equal to its maximum value. This maximum value is given by Eq. A19 in the Appendix.

Since the objective function of problem 33 is minus the sum of $C_2^i(\tau_{i-1}, \tau_i)$ and when inequality 36 holds there is the possibility of having $\boldsymbol{\tau} \ge \mathbf{1}$ for which (a) the equality constraint is satisfied (Eq. 34), (b) each $C_2^i(\tau_{i-1}, \tau_i)$ obtains its maximum possible value, and (c) the inequality constraints (Eq. 35) are automatically satisfied, it is clear that the optimum is achieved for $\tau_i \ge 1$ *for all* i $\in [1, N^k]$.

Finally, since there are infinite many vectors $\boldsymbol{\tau} \ge \mathbf{1}$, which satisfy the equality and inequality constraints of the primal problem (Eq. 32), while the value of the objective function does not change, it is obvious that statement (b) of the proposition also holds.

Proposition 2: *If inequality 37 holds (that is, for $7 \le N^k \le 65$), and if there exists a local solution to the primal problem (Eq. 32), then it must satisfy: $\boldsymbol{\tau} \in \boldsymbol{S} = \{\boldsymbol{\tau}: h(\boldsymbol{\tau}) = 0, \boldsymbol{g}(\boldsymbol{\tau}) \le \mathbf{0}$, and $\boldsymbol{\tau} < \mathbf{1}\}$.*

Proof: Since we assumed that Eq. 32 has a local solution, then conditions 38–40 of theorem 1 must be satisfied (KKT gradient conditions, KKT complementarity conditions, nonnegativity of inequality constraint Lagrange multipliers). Also, the equality constraint (Eq. 34) and the inequality constraints (35) must also be satisfied.

We will prove the proposition by contradiction: Suppose that: $\exists j, 1 \le j \le N^k$ such that: $\tau_j \ge 1$. Using Eqs. 33–35, we see that the corresponding (jth) equation of the system of KKT gradient conditions (Eq. 38) will read

$$-\frac{\partial C_2^j(\tau_{j-1},\tau_j)}{\partial \tau_j} - \frac{\partial C_2^{j+1}(\tau_j,\tau_{j+1})}{\partial \tau_j} + \lambda \frac{r_p^j}{E_1^j} \cdot (x_{\max} - x_{\min}) - \nu_j^2 = 0 \qquad \text{(B1)}$$

Then, for $\tau_j \ge 1$ and according to what we have shown in Appendix A, the concentration of diblock copolymers produced during the jth and $(j+1)$th stages of the process are both independent of τ_j, that is,

$$\frac{\partial C_2^j(\tau_{j-1},\tau_j)}{\partial \tau_j} = \frac{\partial C_2^{j+1}(\tau_j,\tau_{j+1})}{\partial \tau_j} = 0 \qquad \text{(B2)}$$

Moreover, due to the complementarity conditions (Eq. 39) that we assumed they hold, and since, for $\tau_j \ge 1$, the strict inequality $g_j^k(\tau_j) = \tau_{\min} - \tau_j < 0$ holds, we must also have that

$$\mu_j = \nu_j^2 = 0 \qquad \text{(B3)}$$

Substituting Eqs. B2 and B3 into B1 and using the fact that $r_p^j/E_1^j \cdot (x_{\max} - x_{\min})$ is always a positive constant, we find that for the single equality constraint Lagrange multiplier we have

$$\lambda = 0 \tag{B4}$$

Since inequality 37 holds, there must exist at least one dimensionless switching time that is strictly less than 1, in order for the equality constraint 34 to be satisfied, that is, $\exists i, 1 \le i \le N^k$ such that: $\tau_i < 1$. Then using Eq. B4, the corresponding ith equation of the KKT gradient conditions will read

$$-\frac{\partial C_2^i(\tau_{i-1}, \tau_i)}{\partial \tau_i} - \frac{\partial C_2^{i+1}(\tau_i, \tau_{i+1})}{\partial \tau_i} - \nu_i^2 = 0 \tag{B5}$$

As we have shown in Appendix A, for $\tau_i < 1$ we have that

$$-\frac{\partial C_2^i(\tau_{i-1}, \tau_i)}{\partial \tau_i} < 0$$

$$-\frac{\partial C_2^{i+1}(\tau_i, \tau_{i+1})}{\partial \tau_i} < 0 \tag{B6}$$

Since also conditions 40 hold: we have: $-\nu_i^2 < 0$. Therefore, it is obvious from Eq. B6 that Eq. B5 cannot be satisfied. Thus, it is impossible for the KKT gradient conditions (Eq. 38) to be satisfied. This is a contradiction, since we assumed that there exists a local solution to the optimization problem (Eqs. 32), and, hence, the KKT gradient conditions (Eq. 38) must hold. Consequently, our original assumption (that is, $\exists j, 1 \le j \le N^k$ such that: $\tau_j \ge 1$) is wrong. Hence, if a local solution to problem 32 exists, then we must have: $\boldsymbol{\tau} < \mathbf{1}$. Since we have also assumed that the equality and inequality constraints (Eqs. 34, 35, respectively) hold, we conclude that if a local solution to the primal problem (Eq. 32) for $7 \le N^k \le 65$ exists then: $\tau \in \boldsymbol{S} = \{\boldsymbol{\tau}: h(\boldsymbol{\tau}) = 0,\ \boldsymbol{g}(\boldsymbol{\tau}) \le \mathbf{0}$, and $\boldsymbol{\tau} < \mathbf{1}\}$.

Lemma 2: *The Hessian of the objective function (Eq. 33) $[\nabla^2 f^k(\boldsymbol{\tau})]$ is positive definite $\forall \boldsymbol{\tau} \in \boldsymbol{S}' = \{\boldsymbol{\tau}: \boldsymbol{g}(\boldsymbol{\tau}) \le \mathbf{0}$ and $\boldsymbol{\tau} < \mathbf{1}\}$.*

Proof: Let us use the symbol $\underline{\underline{H}}(\boldsymbol{\tau}) = \nabla^2 f^k(\boldsymbol{\tau})$ for the Hessian matrix of the objective function. We know that $C_2^i(\tau_{i-1}, \tau_i)$ depends only on the current [ith] and previous [$(i-1)$th] dimensionless switching times. This implies that each entry τ_i of $\boldsymbol{\tau}$ influences only the concentrations of diblock copolymer produced during the ith and $(i+1)$th stages of the process $C_2^i(\tau_{i-1}, \tau_i)$, and $C_2^{i+1}(\tau_i, \tau_{i+1})$, respectively. Hence, the $N^k \times N^k$ Hessian matrix is tridiagonal. The diagonal elements are given by the expressions

$$H_{1,1} = -\frac{\partial^2 C_2^2(\tau_1, \tau_2)}{\partial \tau_1^2}$$

$$H_{i,i} = -\left[\frac{\partial^2 C_2^i(\tau_{i-1}, \tau_i)}{\partial \tau_i^2} + \frac{\partial^2 C_2^{i+1}(\tau_i, \tau_{i+1})}{\partial \tau_i^2}\right] \qquad i = 2, \ldots, N^k - 1$$

$$H_{N^k, N^k} = -\frac{\partial^2 C_2^{N^k}(\tau_{N^k-1}, \tau_{N^k})}{\partial \tau_{N^k}^2} \tag{B7}$$

The elements of the lower diagonal are given by the expressions

$$H_{i,i-1} = -\frac{\partial^2 C_2^i(\tau_{i-1}, \tau_i)}{\partial \tau_i \partial \tau_{i-1}} \quad \forall i = 2, \ldots N^k \tag{B8}$$

while the elements of the upper diagonal are given by the expressions

$$H_{i,i+1} = -\frac{\partial^2 C_2^{i+1}(\tau_i, \tau_{i+1})}{\partial \tau_i \partial \tau_{i+1}} \quad \forall i = 1, \ldots N^k - 1 \tag{B9}$$

From Eqs. B8 and B9, it is clear that the Hessian matrix is symmetric.

As we have shown in Appendix A, both

$$\frac{\partial^2 C_2^i(\tau_{i-1}, \tau_i)}{\partial \tau_{i-1}^2} \quad \text{and} \quad \frac{\partial^2 C_2^i(\tau_{i-1}, \tau_i)}{\partial \tau_i^2}$$

are strictly negative for each pair τ_i, $\tau_{i-1} \in [0.1, 1)$. Hence, from Eq. B7 and since $\boldsymbol{\tau} < \mathbf{1}$, we have that all diagonal elements of the Hessian matrix are strictly positive $\forall \boldsymbol{\tau} \in \boldsymbol{S}'$. Moreover, since

$$\frac{\partial^2 C_2^i(\tau_{i-1}, \tau_i)}{\partial \tau_i \partial \tau_{i-1}}$$

are non-negative for each pair τ_i, $\tau_{i-1} \in [0.1, 1)$, as shown in Appendix A, we conclude that all off-diagonal elements of the Hessian matrix are non-positive $\forall \boldsymbol{\tau} \in \boldsymbol{S}'$.

Moreover, consider the quotient defined as follows

$$q_i = |H_{i,i}| - |H_{i,i-1}| - |H_{i,i+1}| \qquad i = 1, \ldots, N^k \tag{B10}$$

Substituting Eqs. B7, B8 and B9 into B10 we get

$$q_1 = \left| -\frac{\partial^2 C_2^2(\tau_1, \tau_2)}{\partial \tau_1^2} \right| - \left| -\frac{\partial^2 C_2^2(\tau_1, \tau_2)}{\partial \tau_1 \partial \tau_2} \right|$$

$$q_i = \left| -\left(\frac{\partial^2 C_2^i(\tau_{i-1}, \tau_i)}{\partial \tau_i^2} + \frac{\partial^2 C_2^{i+1}(\tau_i, \tau_{i+1})}{\partial \tau_i^2} \right) \right| - \left| -\frac{\partial^2 C_2^i(\tau_{i-1}, \tau_i)}{\partial \tau_i \partial \tau_{i-1}} \right| - \left| -\frac{\partial^2 C_2^{i+1}(\tau_i, \tau_{i+1})}{\partial \tau_i \partial \tau_{i+1}} \right| \qquad i = 2, \ldots N^k - 1$$

$$q_{N^k} = \left| -\frac{\partial^2 C_2^{N^k}(\tau_{N^k-1}, \tau_{N^k})}{\partial \tau_{N^k}^2} \right| - \left| -\frac{\partial^2 C_2^{N^k}(\tau_{N^k-1}, \tau_{N^k})}{\partial \tau_{N^k} \partial \tau_{N^k-1}} \right| \tag{B11}$$

From Eq. B11, we clearly see that for $i = 2, \ldots, N^k - 1$, quotient q_i depends on three dimensionless switching times, whereas for $i = 1$ and $i = N^k$ it depends on just two. As we have shown in Appendix A, both second derivatives are strictly greater in magnitude than the corresponding second mixed derivative, for each pair τ_i, $\tau_{i-1} \in [0.1, 1)$. Due to this fact, and since all diagonal elements are always strictly positive and all off-diagonal elements are always non-positive $\forall \boldsymbol{\tau} \in \boldsymbol{S}'$, from Eq. B10, it is obvious that

$$q_1(\tau_1, \tau_2) > 0 \quad \forall \tau_1, \tau_2 \in [0.1, 1)$$

$$q_i(\tau_i, \tau_{i-1}, \tau_{i+1}) > 0 \quad \forall \tau_i, \tau_{i-1}, \tau_{i+1} \in [0.1, 1) \quad \text{and } \forall i = 2, \ldots, N^k - 1$$

$$q_{N^k}(\tau_{N^k-1}) > 0 \quad \forall \tau_{N^k}, \tau_{N^k-1} \in [0.1, 1) \tag{B12}$$

Thus, the Hessian matrix is strictly diagonally dominant $\forall \boldsymbol{\tau} \in \boldsymbol{S}'$.

According to Gershgorin's theorem (Saad, 1996), any eigenvalue of a matrix is located in one of the closed discs of the complex plane centered at the diagonal elements of the matrix and having a radius equal to the magnitude of the sum of all corresponding off diagonal elements. Since, in this case, the Hessian matrix is strictly diagonally dominant, the radius of any of these closed discs cannot exceed or become equal to the magnitude of the corresponding diagonal element. Since also the diagonal elements are all strictly positive, we conclude that no eigenvalue can become negative or zero. Therefore, the Hessian matrix $\underline{\underline{H}}(\boldsymbol{\tau}) = \nabla^2 f^k(\boldsymbol{\tau})$ is positive definite $\forall \boldsymbol{\tau} \in S'$.

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