Modelling the Cell Cycle

Gary Chaffey

Thesis submitted to the University of Surrey
for the degree of Doctor of Philosophy

Department of Mathematics
University of Surrey
Guildford GU2 7XH, United Kingdom

Copyright © 2014 by Gary Chaffey. All rights reserved.
E-mail address: g.chaffey@surrey.ac.uk
Scientific Abstract

This thesis may be divided into two related parts. The first of which considers a population balance approach to modelling a population of cells, with particular emphasis on how the cells pass between the $G_1$ and $S$ phases of the cell cycle. In the second part of the thesis a model is described which combines a cell cycle model with a simple Pharmacokinetic/Pharmacodynamic (PKPD) drug model. This model is then discussed in detail.

Knowledge of how a population of cancerous cells progress through the cell cycle is vital if the population is to be treated effectively, as treatment outcome is dependent on the phase distributions of the population. Estimates on the phase distribution may be obtained experimentally however the errors present in these estimates may effect treatment efficacy and planning. In this thesis mathematical models are used to explore the factors that effect the phase distributions of the population.

In this thesis it is shown that two different transition rates at the $G_1$-$S$ checkpoint provide a good fit to a growth curve obtained experimentally. However, the different transition functions predict a different phase distribution for the population, but both lying within the bounds of experimental error. Since treatment outcome is effected by the phase distribution of the population this difference may be critical in treatment planning.

Using an age-structured population balance approach the cell cycle is modelled with particular emphasis on the $G_1$-$S$ checkpoint. By considering the probability of cells transitioning at the $G_1$-$S$ checkpoint, different transition functions are obtained. A suitable finite difference scheme for the numerical simulation of the model is derived and shown to be stable. The model is then fitted using the different probability transition functions to experimental data and the effects of the different probability transition functions on the model's results are discussed.
In contrast to the population balance approach a more simplistic compartmental model is also considered. This model results in a system of linear ordinary differential equations which, under specific circumstances may be solved analytically. It is shown that whilst not as accurate as the population balance model this model provides an adequate fit to experimental data with the results for the total cell population lying within the bounds of experimental error.

The ODE compartment model is combined with a simple PKPD model to allow a detailed analysis of the equations for this combined model to be undertaken for different drug-cell interactions. These results are then discussed.

As a tumour grows many of the cells receive oxygen and nutrients from blood vessels formed within the tumour, these provide a less than ideal supply, resulting in areas that are well perfused, hypoxic and necrotic. In hypoxic regions the lack of oxygen and nutrients limit the cells’ growth by increasing their cycle time and also reducing the effects of radiation and chemotherapy. In the conclusion of this thesis the idea of separating a tumour into three regions, normoxic, hypoxic and necrotic is discussed. Each of these regions may then be modelled using three coupled compartments, each of which contain a cell cycle model, modelled using a set of ordinary differential equations. Additionally, the interaction of a simple (PKPD) drug model with these populations of cells may be considered.

Keywords and AMS Classification Codes: Cell Cycle, phase distribution, age structured mathematical model, mathematical modelling, 35Q92, 92C37
Lay Summary

Cancer tumours are comprised of a heterogeneous population of cells differing in age, size and DNA content. Such populations may be modelled mathematically using a population balance framework giving a set of partial differential equations for the cell population density. Such a model may then be modified to account for external factors affecting the population such as nutrient deprivation, radiation treatment or cytotoxic drugs allowing for a greater understanding of how the carcinogenesis process may be controlled.
Declaration

This thesis and the work to which it refers are the results of my own efforts. Any ideas, data, images or text resulting from the work of others (whether published or unpublished) are fully identified as such within the work and attributed to their originator in the text, bibliography or in footnotes. This thesis has not been submitted in whole or in part for any other academic degree or professional qualification. I agree that the University has the right to submit my work to the plagiarism detection service Turnitin UK for originality checks. Whether or not drafts have been so-assessed, the University reserves the right to require an electronic version of the final document (as submitted) for assessment as above.

Signed:

Date:

Copyright ©2014 by Gary Chaffey.

The copyright in text of this thesis rests with the author. No substantial quotations from it should be published without the author’s prior written consent and information derived from it should be acknowledged.
Acknowledgements

Firstly, I would like to thank my supervisors Dr Anne Skeldon, Dr David Lloyd and Professor Norman Kirkby for the guidance, help and advice they have given me over the course of my PhD. Their input, encouragement and words of wisdom have proven invaluable. A special thank you also goes to Professor Karen Kirkby for her guidance and support in the first year of my Doctoral Training Centre PhD. I would also like to thank Dr Carina Dunlop and Dr Sara Maad Sasane for their helpful comments and suggestions in the regular reading group meetings.

I gratefully acknowledge the financial support of the Doctoral Training Centre supported by the EPSRC.

A huge heartfelt thank you to my fiancée Natasha whose faith in me has helped me to stay focussed and complete this PhD. Thank you to my daughters Lucy and Amy who have tolerated me being stressed so well and whose witty and sarcastic comments about me working in the garden shed have kept me grounded and sane. Finally, thank you to my son Theodore, who has provided me with lots of distractions and sleepless nights in the last few months of my PhD!
In Loving Memory of my Father
Contents

Scientific Abstract i

Lay Summary iii

Acknowledgements v

List of Figures 1

List of Tables 4

1 Introduction 6

1.1 The Cell Cycle 6

1.2 Overview 8

1.2.1 Chapter 2: Cell Cycle Models 9

1.2.2 Chapter 3: Validation and Comparison of New Ordinary Differential Equation and Partial Differential Equation Models 9

1.2.3 Chapter 4: Integral Equations for an Age Structured Population Balance Model 10

1.2.4 Chapter 5: Pharmacokinetic and Pharmacodynamic Models 11

1.2.5 Chapter 6: A Cell Cycle Model with Drug Interaction 11

1.2.6 Chapter 7: Conclusion and Further Work 12

2 Cell Cycle Models 13

2.1 Ordinary Differential Equation Models 14

2.1.1 Steel’s Formulae 14

2.1.2 Structured ODE Models 17

2.1.3 Molecular ODE Models 19

2.2 Partial Differential Equation Models 20

2.2.1 Population Balance Models 20

2.3 Summary of Existing Cell Cycle Models 37
3 Validation and Comparison of New Ordinary Differential Equation and
Partial Differential Equation Models
   3.1 Structured Ordinary Differential Equation Model .......................... 41
      3.1.1 Comparison of Results Obtained Using Steel's Formulae and the Four
              Compartment ODE Model ........................................... 45
   3.2 A Simplified Age Structured Partial Differential Equation Model ........ 49
   3.3 Transition Functions ....................................................... 56
      3.3.1 Derivation of Transition Functions .................................. 57
      3.3.2 Constant Transition Function ...................................... 59
      3.3.3 Quadratic Transition Function ..................................... 59
      3.3.4 Sigmoidal Transition Function ..................................... 61
      3.3.5 New Sigmoidal Transition Function ................................ 66
   3.4 Numerical Scheme ............................................................. 68
      3.4.1 Stability of the Numerical System .................................. 70
   3.5 PDE Model Validation and the Effect of The Transition Function .......... 74
      3.5.1 The Effect of the Transition Function .............................. 77
   3.6 Ordinary Differential Equation Model Validation and Comparison of Ordinary
       Differential Equation and Partial Differential Equation Models .......... 83
   3.7 Cell Cycle Model Summary .................................................. 86

4 Integral Equations for an Age Structured Population Balance Model ....... 88
   4.1 Construction of the Population Balance Model ............................. 89
   4.2 Solution of the PDE System ................................................ 91
      4.2.1 Solution for Compartment A ......................................... 91
      4.2.2 Solution for Compartment B ......................................... 92
      4.2.3 Solution for Compartment C ......................................... 92
      4.2.4 Solution for the D Compartment ................................... 97
   4.3 Equations for $M(t)$ ......................................................... 97
   4.4 Equations for $P(t)$ ......................................................... 99
      4.4.1 Equations for $P_A(t)$ ............................................... 99
      4.4.2 Equations for $P_B(t)$ ............................................... 100
      4.4.3 Equations for $P_C(t)$ ............................................... 101
      4.4.4 Equations for $P_D(t)$ ............................................... 107
   4.5 Integral Equations Summary ................................................. 108


5 Pharmacokinetic and Pharmacodynamic Models

5.1 Mathematical Modelling of Pharmacokinetics and Pharmacodynamics  110
  5.1.1 Simple Intravenous Models  112
  5.1.2 Simple Absorption Models  114
  5.1.3 Periodic doses  119
5.2 Pharmacokinetic and Pharmacodynamic Model Summary  120

6 A Cell Cycle Model with Drug Interaction

6.1 Outline of Model  122
  6.1.1 Types of Drug Interaction  126
6.2 The Effect of a Cytotoxic Drug Interaction on A Population of Cells  127
  6.2.1 Non-Dimensionalisation of the Equations  128
  6.2.2 Case I : \( \epsilon \ll 1 \)  130
  6.2.3 Case II : \( \epsilon = \mathcal{O}(1) \)  154
  6.2.4 Case III : \( \epsilon \to \infty \)  154
6.3 The Effect of a Cytostatic Drug Interaction on A Population of Cells  155
  6.3.1 Non-Dimensionalisation of the Equations  156
  6.3.2 Case I : \( \epsilon \ll 1 \)  158
  6.3.3 Case II : \( \epsilon = \mathcal{O}(1) \)  165
  6.3.4 Case III : \( \epsilon \to \infty \)  166
6.4 Comparison of the Effects of Cytotoxic and Cytostatic Drug Interactions  167
6.5 ODE Drug Interaction Summary  168

7 Conclusion and Further Work

7.1 Summary  170
  7.1.1 Summary of Chapter 2 : Cell Cycle Models  170
  7.1.2 Summary of Chapter 3 : Validation and Comparison of New Ordinary
      Differential Equation and Partial Differential Equation Models  171
  7.1.3 Summary of Chapter 4 : Integral Equations for an Age Structured Popu-
      lation Balance Model  172
  7.1.4 Summary of Chapter 5 : Pharmacokinetic and Pharmacodynamic Models  173
  7.1.5 Summary of Chapter 6 : A Cell Cycle Model with Drug Interaction  173
7.2 Conclusion .................................................. 175
7.3 Further Work .................................................. 175
  7.3.1 Overcrowding .......................................... 175
  7.3.2 Periodic Dosing .......................................... 178
  7.3.3 Multiple Compartments .................................. 179
  7.3.4 Competition ............................................. 182

A Modified Sigmoidal Transition Function, Accounting for the Effects of Limited Nutrient Availability 183

B Construction of the Time Step Matrix 185

C An Alternative Approach for the Proof of Stability of the Numerical Scheme 188

D Change of Order of Integration for $P_C(t)$ 190

E A Check of the Numerical Approximations for the Solutions to ODEs 191
  E.1 Second Order ODE Solution Comparison ...................... 191
  E.2 Fourth Order ODE Solution Comparison ...................... 195

F Case I for a Cytotoxic Drug-Cell Cycle Model When Phase Rate Constants Are Not Equal 197
  F.1 Non-dimensionalisation of the Equations ..................... 198
  F.2 Obtaining an Approximate Solution .......................... 201

References .................................................. 210

Scientific Contribution ........................................ 216
List of Figures

1.1 Illustration of a Cell Cycle Model. ............................................. 8

2.1 Illustration of the Cell Cycle Model Described in [3]. ..................... 26

2.2 Illustration of the CelCyMUS Cell Cycle Model. ............................. 30

3.1 Compartimental Representation of the Cell Cycle. .......................... 42

3.2 Growth Curves for the Number of Cells in Different Phases as a Function of Time when $k_i = 1$, $i \in \{1, 2, 3, 4\}$ and $N_0 = 10^6$. ................. 47

3.3 Fraction of the Total Population of Cells in Different Phases as a Function of Time when $k_i = 1$, $i \in \{1, 2, 3, 4\}$ and $N_0 = 10^6$. ................. 47

3.4 Illustration of a Simplified Cell Cycle Model. ............................... 51

3.5 Probability Distribution of Transition, $f(\tau)$ Showing the Probability that a Cell of Age $\tau$ has not yet Transitioned (Shaded Region) and the Probability a Cell of Age $\tau$ will Transition in the Time Interval $\tau$ to $\tau + \delta \tau$ (Dark Region). 57

3.6 Sketch of a Quadratic Cumulative Probability Distribution Function for an Arbitrary Time for the Transition Function Presented in [22]. .......... 60

3.7 Sketch of the Quadratic Cumulative Probability Distribution Function for an Arbitrary Time Presented in [22]. ................................. 62

3.8 A Plot of $F(\tau_{G_{1b}})$ as Defined in Equation (3.61) with $C_c(t, \tau_{G_{1b}}) = R\tau_{G_{1b}}$ Where $R$ is a Constant for $R = 0.1$, $d_{G_{1b}} = 1$ and $\alpha_{G_{1b}} = 5$. .............................. 64

3.9 A Plot of $F(\tau_{G_{1b}})$ as Defined in Equation (3.67) for $R = 0.1$, $d_{G_{1b}} = 1$ and $\alpha_{G_{1b}} = 5$. ......................................................... 65
3.10 Illustration of the Two Compartment Model Used in the Stability Analysis of the Numerical Scheme. ........................................ 71

3.11 Growth Curves Produced by Using a Constant Transition Rule (a) and a Sigmoidal Transition Rule (b) Fitted Against Experimental Batch Data Presented in [23]. .................................................. 76

3.12 Proportions of Cells in Each Phase Using a Constant Transition Rule (a) and a Sigmoidal Transition Rule (b). ........................................ 79

3.13 Constant Transition Function (a) With the Corresponding Cumulative Probability of Transition (b) As a Function of \( G_{1b} \) Age. .............. 80

3.14 Sigmoidal Transition Function (a) With the Corresponding Cumulative Probability of Transition (b) As a Function of \( G_{1b} \) Age. .............. 81

3.15 Illustration of a Two Compartment ODE Model. .......................... 84

3.16 Growth Curve Produced from a Two Compartment ODE Model Fitted Against Experimental Batch Data Presented in [23] ..................... 85

4.1 Illustration of a Four Compartment Cell Cycle Model, Including a Quiescent Phase. ............................................................... 89

4.2 Graphical Representation of the Region of Integration for \( P_C(t) \) when \( t < T_A \). 102

5.1 Pharmacokinetic Model Without Absorption. .............................. 111

5.2 Pharmacokinetic Model With Absorption. ................................... 111

5.3 A Sketch of a Typical Plasma Drug Concentration Curve When the Dose is Administered Via Constant Infusion. .............................. 115

5.4 A Sketch of a Typical Plasma Drug Concentration Curve When the Dose is Administered Via an Absorption Process. .......................... 117

5.5 Pharmacokinetic Absorption Model with Loss from Outer Compartment. 118

5.6 Pharmacokinetic Absorption Model With Loss From the Outer Compart- ment and Two Way Drug Diffusion. ........................................ 119

6.1 Four Compartment ODE Cell Cycle Model Incorporating Drug Interaction. 124
6.2 Plot of the Analytical Solution for the Second Order ODE Given in Equation (6.30) for $t \in [0, 5]$ and $\lambda = 5.$  
6.3 Plot of the Analytical Solution for the Second Order ODE Given in Equation (6.30) for $t \in [0, 1]$ and $\lambda = 5.$  
6.4 Plot Showing a Second Order Approximation Together with Actual Solution for the Second Order ODE Given in Equation (6.30).  
6.5 Plot Showing a First Order Approximation Together with Actual Solution for the Fourth Order ODE Given in Equation (6.26) with $D_I = 1$ and $\lambda = 2.151$  
6.6 Plot Showing an Approximation to the Total Cell Population with Cytotoxic Effect with $D_I = 1$ and $\lambda = 2.$  
6.7 Plot Showing an Approximation to the Total Cell Population with Cytotoxic Effect when $D_I = 2$ and $\lambda = 2.$  
6.8 Plot Showing a First Order Approximation Together with Actual Solution for the Fourth Order ODE Given in Equation (6.130) with $D_I = 1$ and $\lambda = 2.163$  
6.9 Plot Showing an Approximation to the Total Cell Population with Cytostatic Effect with $D_I = 1$ and $\lambda = 2.$  
6.10 Plot Showing a First Order Approximation for Both Cytotoxic and Cytostatic Models, Together with the Asymptote Both Models Tend to as $t \to \infty.$  

7.1 Sketch of A Suitable Overcrowding Function for which Cell Growth is Optimal at Moderate Cell Densities.  
7.2 The Effect of Introducing an Overcrowding Function on the Cell Density.  
7.3 A Combined PKPD and Cell Cycle Three Compartment Model.  

D.1 Graphical Representation of the Region of Integration for $P_C(t)$ when $T_C \leq t < T_B.$  

F.1 Comparision of Solutions Obtained Using a First Order Frobenius Technique and Maple’s Numerical Intergrator for a Cytotoxic Drug-Cell Cycle Model where Rate Constants Are Not Equal.
List of Tables

3.1 Parameters from [23] .................................................. 74
3.2 Parameters Fitted to A Two Compartment ODE Model. ............... 84
Chapter 1

Introduction

Cancer tumours are comprised of a heterogeneous population of cells differing in age, size and DNA content. The vulnerability of cells to different treatments varies according to their position in the cell cycle, therefore knowledge of how a population of cells progress through the various phases of the cycle is vital if the population is to be treated effectively. Estimates on the phase distribution may be obtained experimentally however the errors present in these estimates may effect treatment efficacy and planning. If mathematical models are to be used to make accurate, quantitative predictions concerning treatments, whose efficacy is phase dependent, knowledge of the phase distribution is crucial.

1.1 The Cell Cycle

The cell cycle is an ordered set of events that a cell undergoes from its birth until it divides into two daughter cells [1] as depicted in Figure 1.1. In eukaryotic cells the cell cycle may be broken down into four distinct phases, namely $G_1$, $S$, $G_2$ and $M$. After birth, a cell enters the longest of the phases, the $G_1$ (Gap 1) phase, during which the cell takes on nutrients needed to complete the rest of the cycle. Once the cell has absorbed enough nutrients it may proceed round the cell cycle leaving the $G_1$ phase and entering the $S$ (Synthesis) phase. Not all cells leave the $G_1$ phase to enter the $S$ phase, a number of cells enter a quiescent period where they remain viable but leave the cell cycle for a short time, these cells enter the $G_0$ (Gap 0) phase. During the $S$ phase a cell replicates its...
1.1 The Cell Cycle

DNA, at the end of which they have effectively doubled their DNA content. Once DNA synthesis is completed the cell enters the $G_2$ (Gap 2) phase. During the $G_2$ a cell grows in size and prepares for mitosis. Upon leaving $G_2$ the final phase $M$ (Mitosis) is entered. It is during the mitotic phase that the cell divides, producing two daughter cells. Due to the processes involved in cell division, cells in the $M$ phase are especially vulnerable to radiotherapy. It should be noted that the $M$ phase may be broken down further into several sub phases, however this is of no consequence for the cell cycle models discussed in this thesis. The actual length of the cell cycle is variable, this variability mainly occurs in the length of time cells spend in the $G_1$ phase which is governed by the way in which cells ‘transition’ from the $G_1$ phase to the $S$ phase [59]. Once a cell commits itself to DNA synthesis (i.e. enters the $S$ phase) it must continue the cell cycle until division is complete, the ‘transition’ from the $G_1$ phase to the $S$ phase is irreversible. Chemotherapy drugs can be divided into several types, each of which target a specific process within the cell cycle such as RNA synthesis or cell division. Hence the efficacy of many chemotherapy drugs (e.g. [11], [40] and [50]) is dependent on the cell cycle phase. The radiosensitivity of cells is also phase dependent (e.g. [13], [43] and [64]) with cells in the $M$ (mitotic) phase having their chromosomes arranged in a line prior to separation making them particularly sensitive to ionising radiation. Due to the phase dependent nature of chemotherapy drugs and radiotherapy knowledge of how the cells progress through the different phases is crucial.
1.2 Overview

There have been numerous mathematical models developed that describe how populations of cells progress round the cell cycle. Such models vary greatly in complexity from a single ordinary differential equation (ODE) modelling the growth of an entire population\(^2\) to sophisticated multi-scale models that account for intercellular biochemical processes of individual cells and the effect of external factors on the population as a whole [53].

\(^2\)As early as 1798 Thomas Malthus described the exponential growth of a population. By the early twentieth century this concept was being applied to populations of cells.
The outline of this thesis is as follows

1.2.1 Chapter 2 : Cell Cycle Models

In Chapter 2 a detailed literature review of existing cell cycle models is undertaken. Section 2.1 starts with considering a simple ODE model in which the entire population of cells is assumed to be homogeneous. Steel's formulae, which allows information about the age structure of the population to be inferred from a simple ODE model is derived. This derivation is undertaken so that it is clear where the formulae is valid and under what circumstances it may not yield correct results. Structured models, resulting in systems of coupled ODEs are then discussed with a brief review of some existing models that use this approach. Section 2.1 concludes with a review of molecular ODE models, it is then explained why this approach is not considered further.

To account for the intraphase heterogeneity of the population it is necessary to model the population using partial differential equations (PDEs). Whilst there are several different approaches for modelling a population of cells using PDEs the most popular approach is to use a population balance framework. In Section 2.2.1 a detailed review is undertaken of models that use such a framework, resulting in a system of PDEs which describe some of the intrinsic properties of the population such as age or DNA distribution. Chapter 2 concludes with a brief discussion of the existing models highlighting limitations and possible areas for improvement.

1.2.2 Chapter 3 : Validation and Comparison of New Ordinary Differential Equation and Partial Differential Equation Models

In Chapter 3 new ODE and PDE models are formulated. A new PDE model, based upon the CelCyMUS model (22, 23 and 57), is formulated and verified. This new PDE model is then compared against the ODE modelling approach.

In Section 3.1 a new structured ODE model is constructed. This model consists of four compartments, each corresponding to a different phase of the cell cycle. The rate constants, which define the rates at which cells pass between these compartments are chosen to all be unity allowing an analytic solution to be obtained. This model gives an alternative to Steel's formulae for calculating the percentage of cells in each phase, and a
detailed comparison between this result and Steel’s formulae is undertaken. The ODE model presented also allows for a good comparison of ODE and PDE models and forms a general framework which is extended in Chapter 6.

Section 3.2 starts with a brief discussion of simplifications which may be made to the CelCyMUS model, details of which may be found in Section 2.2.1. Using the CelCyMUS model as a starting point a new three compartment, age-structured PDE model is presented.

In Section 3.3 two different approaches for deriving the transition function which describes how cells transition from the $G_1$ phase to $S$ phase are detailed. Constant, quadratic and sigmoidal functions from the literature [6], [22], [23] and [57] are discussed in detail, with respective flaws highlighted. A new, sigmoidal transition function is then presented together with an appropriate non-dimensionalisation consistent with the non-dimensionalisation given in Section 3.2 for the new simplified age-structured model.

A numerical scheme for the new simplified age-structured model is derived in Section 3.4, together with a proof of the scheme’s stability.

Section 3.5 sees the simplified age-structured model with different transition functions compared with experimental data. In Section 3.6 the ODE model framework discussed in Section 3.1 is modified to allow direct comparison with the PDE model described in Section 3.2. The ODE model is then fitted to the same experimental data as that used in Section 3.5 and the ODE and PDE results are compared.

Some of the work in this chapter, in particular Section 3.3 has been published jointly with A. Skeldon, D. Lloyd and N. Kirkby [16].

1.2.3 Chapter 4 : Integral Equations for an Age Structured Population Balance Model

In Chapter 4 the construction of integral equations which may be used for the analysis of the cell population is considered. A four compartment age-structured model, similar to that discussed in Section 3.4, is constructed. Two new functions, $M(t)$ and $P(t)$, representing the number of cells dividing and the total number of cells in the population are introduced. The system is then expressed in terms of integral equations for these two new functions. Once $M(t)$ and $P(t)$ have been found the integral equations for the
population densities are readily derived. It should be noted in this chapter the integral equations are derived and no analysis of the resulting system is undertaken. The integral equation approach has been included to indicate alternative methods for the modelling of the cell population are available.

1.2.4 Chapter 5: Pharmacokinetic and Pharmacodynamic Models

Pharmacokinetics (PK) and pharmacodynamics (PD) are the branches of pharmacology which are concerned with how a drug interacts with a biological environment. Pharmacokinetic/pharmacodynamic (PKPD) modelling is an attempt to model the interaction of a drug with a particular biological environment. Chapter 5 starts by giving a brief overview of how PKPD may be modelled. In the case of pharmacodynamics the way in which this may be incorporated directly into a cell cycle model is discussed. A brief introduction to widely used pharmacokinetic modelling is then undertaken. Drugs administered by intravenous infusion and also extravascularly are considered and simple compartment models described. The idea of multiple periodic doses resulting in an iterative map of ODEs is discussed, but not solved. It is explained how the effects of bioavailability may be included and what effect this would have on the resulting solution. Chapter 5 concludes with a brief discussion of the type of PKPD model which will be used in conjunction with cell cycle models in Chapter 6.

1.2.5 Chapter 6: A Cell Cycle Model with Drug Interaction

In Chapter 6 a model for a population of cells effected by drugs is developed. This model enables a semi-analytical solution to be obtained. The solution may be used to gain a qualitative understanding of the way in which different types of drug-cell interactions effect a given population. Since only a qualitative understanding of the system is sought the ODE model described in Section 3.1 and a simple PKPD model similar to that described in Section 5.1.1 are combined.

Chemotherapy drugs effect a population of cells in two different ways, namely cytostasis and cytotoxicity, therefore it seems sensible to consider two separate models. In Section 6.2 the cell cycle model is modified to include the effects of a cytotoxic drug on the population of cells. The modified system of ODEs are presented and non-dimensionalised.
It is observed that the model may be divided into three categories dependent on the order of one of the parameters of the system, each of these is then analysed.

Section 6.3 follows a similar format to Section 6.2 with the model now modified to include the effects of a cytostatic drug on the population of cells. As this scenario is worked through a number of similarities between the cytotoxic and cytostatic model are noted. In Section 6.4 the cytotoxic and cytostatic models are compared with the chapter concluding with a summary of the findings.

The majority of the work in this chapter is currently being prepared for publication with A. Skeldon, D. Lloyd and N. Kirkby.

1.2.6 Chapter 7: Conclusion and Further Work

The thesis concludes by summarising the main results found, and discussing possible ways in which this work could be extended.
Chapter 2

Cell Cycle Models

There have been a number of mathematical models developed that describe how populations of cells progress round the cell cycle. The simplest of these models consist of ODEs that model the growth kinetics of populations of cells and these are detailed in Section 2.1. Section 2.1 starts with considering a simple ODE model in which the entire population of cells is assumed to be homogeneous. It is shown that by utilising Steel’s Formulae it is possible to obtain some information concerning the proportion of cells in each of the cell cycle phases from such a model. The idea of structured ODEs is then discussed with a brief review of some existing models that use this approach. Section 2.1 concludes with a review of molecular ODE models, it is then explained why this approach is not considered further.

Whilst it is possible to construct a model which provides a good representation of the cell population as a whole using ODEs this approach does not consider the intraphase heterogeneity of the population. For this it is necessary to model the population using a PDE model. In Section 2.2.1 a detailed review is undertaken of models that use a population balance framework, resulting in a system of PDEs that describe some of the intrinsic properties of the population such as age or DNA distribution. It should be noted that the models described in this chapter relate to a closed population of cells. This type of closed \textit{in-vitro} system where there is no flow in or out of the system, for a fixed duration, is often referred to as a batch experiment. This chapter concludes with a brief discussion of the existing models highlighting limitations and possible areas for improvement.
2.1 Ordinary Differential Equation Models

The simplest models for the growth of a population of cells consider the population as homogeneous where all individuals have the same intermitotic time $T_C$. If the only variable of interest is the total number of cells then the total number of cells $N$ is governed by the first order ODE

$$\frac{dN(t)}{dt} = \phi N(t) \quad \text{and} \quad N(0) = N_I,$$

where $N_I$ is the number of cells at time $t = 0$ and $\phi$ is the growth constant. Equation (2.1) has the solution

$$N(t) = N_I e^{\phi t}.$$

If the population at $t = 0$ is given by $N_I$ then at time $t = T_C$ all cells will have undergone exactly one division, thus the population will now be $\alpha N_I$. Therefore

$$\alpha N_I = N_I e^{\phi T_C},$$

or

$$\phi = \frac{\ln \alpha}{T_C}.$$

2.1.1 Steel’s Formulae

From a single ODE model for the cell cycle it is possible to obtain further information such as the proportion of cells in each of the phases. This information is useful not only in validating structured ODE models such as that described in Section 3.1 but is also used to obtain parameters for some PDE models such as [3]. Specifically, in [60] it is shown that if the relative phase lengths are known then it is possible to calculate the proportion of cells in each phase of the cell cycle. The results presented in [60] are now reproduced as they provide a useful insight into how further information may be obtained from different ODE models.

Consider an individual cell. If when it divides there are $\alpha$ daughter cells then there is an increase of $\alpha - 1$ cells to the population. Therefore, the fraction of cells produced, at time $t$, $N(t)\alpha$, which are additional, $N(t)(\alpha - 1)$, is given by

$$\frac{N(t)(\alpha - 1)}{N(t)\alpha} = \frac{\alpha - 1}{\alpha}.$$

(2.5)
The growth rate is the fraction of new cells, that are additional, multiplied by the rate at which cells begin a new cycle, \( K \). Since the growth rate is given by \( \phi N(t) \) it follows that

\[
\frac{\alpha - 1}{\alpha} K = \phi N(t).
\]  

(2.6)

Thus, the rate at which cells begin a new cycle is given by

\[
K = \frac{\alpha}{\alpha - 1} \phi N(t).
\]  

(2.7)

Assuming there is no death part way round the cycle the number of cells (the cell density) of age \( \in [\tau, \tau + \delta\tau] \) is given by \( \frac{\alpha}{\alpha - 1} \phi N(t - \tau) \delta\tau \), this is the rate at which cells start a new cycle at time \( t - \tau \). However, from Equation (2.2),

\[
N(t - \tau) = N(t)e^{\phi(t - \tau)},
\]

\[
= N(t)e^{\phi\tau}e^{-\phi\tau},
\]

\[
= N(t)e^{-\phi\tau}.
\]  

(2.8)

Therefore the number of cells whose age is \( \in [\tau, \tau + \delta\tau] \) is given by \( \frac{\alpha}{\alpha - 1} \phi N(t)e^{-\phi\tau} \delta\tau \). Upon normalising the cell density, the probability density function for cells in terms of their age \( P(\tau) \), is obtained

\[
P(\tau) = \frac{\alpha}{\alpha - 1} \phi e^{-\phi\tau}.
\]  

(2.9)

In order to obtain information about the age structure of the population it is necessary to divide the cell cycle into two parts \( \chi \) and \( \psi \) whose durations are \( T_{\chi} \) and \( T_{\psi} \) respectively, such that \( T_{\chi} + T_{\psi} = T_{C} \), then the proportion of cells in the \( \psi \) section is given by

\[
P_{\psi} = \frac{\alpha}{\alpha - 1} \phi \int_{T_{\chi}}^{T_{\chi} + T_{\psi}} e^{-\phi\tau} d\tau,
\]

\[
= \frac{\alpha}{\alpha - 1} \left( e^{-\phi T_{\chi}} - e^{-\phi(T_{\chi} + T_{\psi})} \right),
\]

\[
= \frac{\alpha}{\alpha - 1} \left( e^{-\phi T_{\chi}} - e^{-\phi T_{C}} \right).
\]  

(2.10)

From Equation (2.4) \( \alpha \) may be expressed in terms of \( \phi \) and \( T_{e} \) as \( \alpha = e^{\phi T_{C}} \). Using this
2.1 Ordinary Differential Equation Models

together with the fact that \( T_\chi + T_\psi = T_C \) means Equation (2.10) may be re-written as

\[
P_\psi = \frac{\alpha}{\alpha - 1} \left\{ e^{\phi T_\psi} - \frac{1}{\alpha} \right\},
\]

\[
= \frac{1}{\alpha - 1} \left\{ e^{\phi T_\psi} - 1 \right\}.
\]

By employing Equation (2.11) it is now possible to determine the proportion of cells in the \( \psi \) part of the cycle if the duration, \( T_\psi \), is known.

It should be noted that it is often difficult to determine experimentally the difference between cells in the \( G_2 \) and \( M \) phases thus these two phases are often grouped together, and denoted as \( G_2M \). Let the \( \chi \) section of the cycle contain the \( G_1 \) and \( S \) phases, with the \( G_2 \) and \( M \) phases being contained in \( \psi \) then

\[
P_{G_2M} = \frac{1}{\alpha - 1} \left\{ e^{\phi T_{G_2M}} - 1 \right\}.
\]  

(2.12)

If at the end of the cell cycle every cell divides exactly into two daughter cells then \( \alpha = 2 \).

By setting \( \alpha = 2 \) and substituting for \( \phi \) from Equation (2.4) this expression becomes

\[
P_{G_2M} = e^{\frac{T_{G_2M}}{T_C} \ln 2} - 1,
\]

\[
= 2^{\frac{T_{G_2M}}{T_C}} - 1.
\]  

(2.13)

Equation (2.13) may be re-arranged to make the duration of \( G_2M \), \( T_{G_2M} \) the subject, i.e.

\[
\ln \left( P_{G_2M} + 1 \right) = \frac{T_{G_2M}}{T_C} \ln 2,
\]

\[
T_{G_2M} = T_C \frac{\ln \left( P_{G_2M} + 1 \right)}{\ln 2}.
\]  

(2.14)

To calculate a similar expression for the \( G_1 \) phase it is necessary to redefine \( \chi \) and \( \psi \) such that the \( \chi \) section only contains the \( G_1 \) phase and the rest of the cycle is contained in \( \psi \).

This gives

\[
P_{S+G_2M} = \frac{1}{\alpha - 1} \left\{ e^{\phi T_{S+G_2M}} - 1 \right\},
\]

\[
= e^{\ln 2 \frac{T_{S+G_2M}}{T_C}} - 1,
\]

\[
= 2^{\frac{T_{S+G_2M}}{T_C}} - 1.
\]  

(2.15)
2.1 Ordinary Differential Equation Models

Proceeding as before yields

\[ T_{S+G_2M} = T_C \frac{\ln(P_{S+G_2M} + 1)}{\ln 2}. \quad (2.16) \]

From Equations (2.13) and (2.15) it follows that the proportion of cells in the S phase, \( P_S \), is given by

\[ P_S = P_{S+G_2M} - P_{G_2M}; \]

\[ = 2 \frac{T_{S+G_2M}}{T_C} - 2 \frac{T_{G_2M}}{T_C}. \quad (2.17) \]

In a similar manner if the duration of the S phase is required and the proportions of cells in the phases are known then Equations (2.14) and (2.16) lead to

\[ T_S = T_{S+G_2M} - T_{G_2M}; \]

\[ = T_C \frac{\ln(P_{S+G_2M} + 1)}{\ln 2} - T_C \frac{\ln(P_{G_2M} + 1)}{\ln 2}, \]

\[ = \frac{T_C}{\ln 2} \{ \ln(P_{S+G_2M} + 1) - \ln(P_{G_2M} + 1) \}. \quad (2.18) \]

Clearly,

\[ P_{G_1} = 1 - P_S - P_{G_2M}; \quad (2.19) \]

and

\[ T_{G_1} = T_C - T_S - T_{G_2M}. \quad (2.20) \]

Thus, if the durations of the phases \( G_1, S \) and \( G_2M \) are known and the population is assumed to be progressing through the cell cycle at a uniform rate then it is possible to obtain the proportion of cells in each phase by using Equations (2.13), (2.17) and (2.19). Conversely, if the proportions are known then the durations for the phases may be obtained using Equations (2.14), (2.18) and (2.20). These results are referred to hereafter as ‘Steel’s formulae’ [60] and are used in Section 3.1 and [3].

2.1.2 Structured ODE Models

By considering different properties of the population of cells it is possible to create a simple, structured model which includes some of the cellular properties. Early examples
of this type of structured model may be found in [54] and [62], both of which contain a good overview of early structured ODE models. In both [54] and [62] the population of cells is considered as a ‘biomass’ comprising of two distinct mass compartments, one relating to the nucleic acids and the other the rest of the active biomass, i.e. mainly proteins. In addition to the biomass the substrate and an inhibitor to growth are also modelled using ODEs. By using a compartmental approach for the biomass, external interactions with the population on a ‘compartment’ specific basis, such as the effect of limited nutrient availability on cell progression,\(^1\) may be included. This is a major advantage over the simple unstructured model described by Equation (2.1). Both [54] and [62] introduce a bottleneck to take into account the growth limiting effects of limited nutrient availability.

The idea of a structured compartment model is extended in [52] where a five compartment model is presented. Each of the five compartments in this model represents a phase in the cell cycle with an additional quiescent phase \(G_0\) being appended to the main four cycling phases, \(G_1, S, G_2\) and \(M\). In this model it is assumed that cells may enter the quiescent \(G_0\) phase from the \(G_1\) phase if a cell attaches to a regulatory molecule. This molecule may in turn be released from the cell allowing the cell to return to the active \(G_1\) phase. This regulatory molecule is assumed to be excreted by all cells; the concentration and degradation of these molecules in the substrate is also accounted for within the model. This model also considers a linear loss of cells from each of the compartments. The resulting system of ODEs is then studied both analytically and with the aid of numerical simulations. A number of equilibrium scenarios are found for differing parameter values. Plantadosi \textit{et al.} then postulate the possible effects of specific external agents, specifically oncology treatments, on the model, however no analysis of this is undertaken.

Motivated by the idea of a structured ODE approach a new four compartment model, where each compartment represents one of the active cell cycle phases, is detailed in Section 3.1.

\(^1\)The effects of which may be more pronounced in a particular part of the cycle.
2.1 Ordinary Differential Equation Models

2.1.3 Molecular ODE Models

An alternative approach to modelling the population of cells using compartmental models such as those described in Section 2.1.2 is to consider an individual cell and model the internal biochemical processes [46]. This type of model is based upon modelling the key processes within the regulatory network of the cell, particularly proteins (such as cyclin) and enzymes (such as Cdc2) concerned with cell maturity.

Even a simplistic model of this type requires a system of nonlinear ODEs with a large number of parameters\(^2\). This type of modelling may be extended to include further detail on the regulatory network [47], [48] and [49]. It is possible to obtain some information from these models using analytical techniques [48], however to fully study the complex systems of nonlinear ODEs governing the internal processes numerical techniques are required. Such models have been effectively used to gain an understanding of the effects of external factors on the cell cycle [49].

Powathil et al. [53] use a multi-scale hybrid modelling approach to combine a molecular ODE model with a cellular automaton approach to simulate a population of cancerous cells and the effects of chemotherapy treatments on this population. The time dependent nature of phase specific drugs is considered and the resulting effectiveness of treatments is discussed.

Whilst being able to effectively model the internal biochemical processes of a cell is valuable, if a population of cells is to be considered the resulting multi-scale model can only effectively be studied using numerical techniques. The large number of parameters present allow for over fitting of a model to experimental data, limiting the model’s ability to predict further scenarios. Also, the size of the population of cells that can be modelled using this technique is limited due to computational resources. For a large population (e.g. \(10^7\) cells) it is not a viable approach. Smaller scale simulations may provide valuable information about the population dynamics but it is not possible to gain much insight into the model from studying the complex system of governing equations, as such this modelling approach has not been used in this thesis.

\(^2\)For the model presented in [46] there are nine ODEs containing 18 parameters.
2.2 Partial Differential Equation Models

Whilst it is possible to construct a model which provides a good representation of the cell population as a whole using ODEs this approach does not consider the intraphase heterogeneity of the population. For all populations of cells there is some degree of heterogeneity, this is even more pronounced for populations of cancerous cells [37]. To accurately represent the population there would need to be an infinite number of compartments as no two cells will be in exactly the same place in their cycle. Thus it is necessary to formulate a model using a different approach.

There are a number of different approaches for modelling cell population dynamics such as Markov chains [65], but the most common approach is to use a population balance framework, modelled using partial differential equations, to take into account the continuous variability of the cell properties.

The remainder of this section is concerned with population balance models. A brief introduction of the population balance framework is given together with an overview of the most common types of structuring used for a population of cells. The concept of partition functions for mass and DNA structured population balance models is introduced. The lack of the need for a partition function for an age structured model is also discussed. DNA, mass and multi variable models are then reviewed with both a single, generation type approach and a multi cell phase approach being considered. Particular emphasis is placed on the DNA structured framework presented by Basse et al. in [3]. Age structured models are then reviewed with a comprehensive review of the CelCyMUS (Cell Cycle Model University of Surrey) age structured model, which will form the basis of a new PDE model to be discussed in Chapter 3.

2.2.1 Population Balance Models

Many of the partial differential equation models share the same fundamental population balance structure as detailed in [20], [24] and [25]. These models may broadly be grouped in terms of which cellular property is used to structure the model, the main properties used being DNA ([3], [4], [5], [33] and [39]), age ([6], [17], [22], [23], [38], and [57]) and mass ([17], [38] and [41]).

There are advantages of using a DNA or mass structured model in as much as these
quantities may be easily determined experimentally, however such a model contains no information about the age of a particular cell. Consequently, it is possible for cells to remain in one phase of the cycle for an infinite amount of time. By use of an age-structured model it is possible to control the length of time a cell may remain in the cell cycle. This is of particular importance for cells in the \( G_1 \) phase as this is the phase with the largest variability in length, due to the differing availability of nutrients for cells within the population. Another advantage of age structuring is that, if growth rates and nutrient uptake rates for a given cell line are known, it is possible to determine the mass and DNA content of a cell from its age. In contrast, when given the cells DNA content or mass it is not possible to determine a cell’s age as there is not a one-to-one mapping between age and DNA or mass.

Analysis has been undertaken to determine the existence and stability of steady size/age distributions \([10]\) which may occur under specific circumstances using an age-structured model. Population balance models have been used not only on healthy, unperturbed cell lines but also to model the effects of various treatments to cancer cell populations \([4], [6], [12] \) and \([33]\).

**Partition Functions**

For population balance models which do not use the age variable for structuring it is necessary to introduce the concept of a partition function \( P \). When a cell divides into two daughter cells the mass and DNA content of the two new cells is not exactly equal. If the structuring variable for the model is denoted by \( x \) then let \( P(x, x') \delta x \) denote the probability of a cell with property \( x' \) producing a daughter cell with the property lying in \([x, x + \delta x]\). Since the continuity of most cellular properties such as mass is preserved at cell division it follows \( P(x, x') \delta x = P(x' - x, x') \delta x \), i.e. the partition function is symmetric about \( x = \frac{x'}{2} \). Different distributions are possible for the partition function but it has been suggested that a symmetric beta distribution is a sensible choice \([30]\) and \([41]\). In the case of multiple variables being used for the structuring of the model this idea may be extended where \( \mathbf{x} \) is now a vector. If age is being used as the variable for structuring the model then since all cells have an age of zero at division/birth a partition function is not required, thus slightly simplifying the equations governing the model.
DNA, Mass and Multi Variable Structured Models

There are a number of different models that consider a population balance framework using DNA ([3], [4], [5], [33] and [39]) and mass ([10], [17], [38] and [41]) as the structuring variable.

In [38] a population of cells is modelled by considering successive generations of cells. It is assumed that cells in the \((k + 1)\)th generation are offspring of cells in the \(k\)th generation only. The general integro-partial differential equation for cells in the \(k\)th generation was shown to be

\[
\frac{\partial n_k(x, t)}{\partial t} + \nabla \cdot \left( \dot{X}(x)n_k(x, t) \right) = 2 \int \Gamma(x')P(x, x')n_{k-1}(x', t)dx' - \Gamma(x)n_k(x, t). 
\] (2.21)

Where \(n_k\) is the number of cells in the \(k\)th generation, \(x\) is the state vector (i.e. the variable(s) which is being used to structure the model), \(\dot{X}\) is the rate of change of the state vector, \(P\) is the partition function and \(\Gamma\) is the division rate function. For an age structured model the analytic solution was presented, together with analytic solutions for a mass-structured model, with specific growth functions. A simple multi-dimensional model structured using both age and mass was also discussed. The growth dynamics of a synchronised population was simulated using both age and mass structured approaches with both showing that any synchronicity diminishes with time. This model was constructed using a single term for the total number of cells within each generation \(n_k\), thus despite accounting for the heterogeneity of the population no knowledge about the phase distributions could be easily inferred.

A similar mass-structured, single compartment model was considered in [41]. In this work the population’s growth was linked to the availability of a substrate. Different partition functions and growth rates were analysed and the system was shown to exhibit periodic solutions in certain cases and reach a steady-state in others. Further analysis of the type of solutions which may occur may be found in [17] where overcrowding and mortality are considered. Once again periodic and steady state solutions were found.

A thorough study of different steady size/mass distributions is undertaken in [7], [8], [9] and [10]. Begg et al. discuss, in detail, the existence of steady distributions for both an age structured and an age-size structured model. It is shown that for an age structured model with periodic birth and death coefficients that when a strictly positive periodic solution exists it is a global attractor. Conditions sufficient for the existence of such a
periodic solution are also presented. A multi compartment age structured model is also considered, but purely from a theoretical view and no attempt is made to validate the model against experimental data. The work also touches on extending this approach to a multi compartment age-size structured model.

**DNA Structured Model**

A DNA structured framework has been used to study both healthy, unperturbed cell lines but also to model the effects of various treatments on populations of cancer cells ([4], [6], [12] and [33]). Since DNA structuring is one of the more common population balance frameworks used for modelling cell populations this is now considered in more detail. The majority of the models using a DNA framework share the same general properties, so only the model presented by [3] will be considered.

This model is described fully by Basse et al. in [3]. The cell cycle is modelled using the four phases, $G_1$, $S$, $G_2$ and $M$, as compartments. The transition between the $G_1$ to $S$ phases, $G_2$ to $M$ phases and $M$ to $G_1$ phases are assumed to occur at constant rates denoted by $k_1$, $k_2$, and $b$ respectively. Modelling these transitions in this way assumes cells can be in the phases $G_1$, $G_2$ and $M$ for an infinite amount of time, i.e. these phases do not have a maximum duration.

During the $S$ phase the cells’ DNA content increases by, on average, a factor of two [3]. In [3] the increase is assumed to be linear and occurs at a constant rate $g$. An additional term accounts for the relative DNA content dispersing slightly. It is argued this dispersion is caused by the observational error in measuring the DNA content, whilst this error should not be ignored inclusion in the model at this stage is not ideal. Regardless of the rationale it is important that some small dispersive term is present as the relative DNA content of cells does not always double exactly [37]. Unlike the other phases the $S$ phase is assumed to be of fixed duration. The model is structured using the relative DNA content $x$; this is used to infer the phases of the cells within the population. Relative DNA content is a popular choice for the structuring of the population balance framework as it may easily be measured using flow cytometry. It is assumed that cells in the $G_1$ phase have an average relative DNA content of one, cells in the $G_2$ and $M$ phases have an average relative DNA content of two and cells in the $S$ phase have an average relative DNA content between one and two.
The equations governing the movement of cells round this model are given by

\[ \frac{\partial G_1(x, t)}{\partial t} = 2^2 bM(2x, t) - (k_1 + \mu_{G_1})G_1(x, t), \quad (2.22) \]

\[ \frac{\partial S(x, t)}{\partial t} = D \frac{\partial^2 S(x, t)}{\partial x^2} - \mu_S S(x, t) - \frac{\partial S(x, t)}{\partial x} + k_1 G_1(x, t) - I(x, t; T_S), \quad (2.23) \]

\[ \frac{\partial G_2(x, t)}{\partial t} = I(x, t; T_s) - (k_2 + \mu_{G_2})G_2(x, t), \quad (2.24) \]

\[ \frac{\partial M(x, t)}{\partial t} = k_2 G_2(x, t) - bM(x, t) - \mu_M M(x, t). \quad (2.25) \]

The first of these equations, Equation (2.22) describes the change in cell density in the

\[ G_1 \] phase of the cycle. The \( 2^2 \) is present as all cells whose DNA content is \( \in [2x, 2x + 2\delta x] \)

(i.e. cells leaving the \( M \) phase) are doubled and mapped to a domain half this size, i.e.

\( [x, x + \delta x] \) (cells entering \( G_1 \) phase). The rate at which cells divide, i.e. the birth rate

is denoted by \( b \). The death rate of cells in the \( G_1 \) phase is given by \( \mu_{G_1} \) and the rate at

which cells transition to the \( S \) phase is \( k_1 \).

Equation (2.23) describes the change in the cell population density for the \( S \) phase of the cycle. Since the cellular DNA does not double exactly during the \( S \) phase a dispersion term, \( D \frac{\partial^2 S(x, t)}{\partial x^2} \), is included. The rate of cells dying in the \( S \) phase is given by \( \mu_S \) and

the cells leaving the \( S \) phase after \( T_S \) hours are represented by the \( I(x, t; T_S) \) term. The \( g \frac{\partial S}{\partial x} (x, t) \) term is necessary to account for the linear increase in the average DNA content for cells whilst they are in the \( S \) phase. The \( I(x, t; T_S) \) term is chosen to ensure that cells

exit the \( S \) phase after a fixed duration \( T_S \). Basse et al. show that \( I(x, t; \tau) \) satisfies

\[ \frac{\partial I(x, t; \tau)}{\partial \tau} = D \frac{\partial^2 I(x, t; \tau)}{\partial x^2} - \mu_S I(x, t; \tau) - \frac{\partial I(x, t; \tau)}{\partial x}, \quad 0 < x < \infty \text{ and } t > \tau > 0, \quad (2.26) \]

with the conditions

\[ I(x, t; 0) = k_1 G_1(x, t - T_S), \quad 0 < x < \infty \text{ and } t \geq 0, \quad (2.27) \]

and

\[ D \frac{\partial I(0, t; \tau)}{\partial x} - gI(0, t; \tau) = 0, \quad t \geq \tau \geq 0. \quad (2.28) \]
2.2 Partial Differential Equation Models

Equations (2.24) and (2.25) describe the change in cell densities in the \( G_2 \) and \( M \) phases respectively. Where \( k_2 \) is the rate cells transition between the \( G_2 \) and \( M \) phases and \( \mu_{G_2} \) and \( \mu_M \) represent the cell death rates in the two corresponding phases.

To complete the description of the cell population it is necessary to also include the initial conditions for the system. These are given by

\[
S(x, 0) = 0, \quad 0 < x < \infty, \quad (2.29)
\]

\[
G_2(x, 0) = 0, \quad 0 < x < \infty, \quad (2.30)
\]

\[
M(x, 0) = 0, \quad 0 < x < \infty, \quad (2.31)
\]

\[
G_1(x, 0) = \frac{a_0}{\sqrt{2\pi}\theta_0^2} \exp\left(-\frac{(x - 1)^2}{2\theta_0^2}\right), \quad 0 < x < \infty. \quad (2.32)
\]

The initial conditions given by Equations (2.29)-(2.31) state no cells start in the \( S \), \( G_2 \) and \( M \) phases. These initial conditions may arise when modelling an \textit{in-vitro} population of cells which has been subject to some form of synchronisation which results in all the cells in the population being in the same phase. Further details of this type of blocking process may be found in [23] and [31]. However, in reality, the whole population will not be synchronised. Equation (2.32) gives the DNA content for cells starting in the \( G_1 \) phase as a Gaussian distribution with relative mean of one, the variance of the distribution is denoted by \( \theta_0^2 \) and the height of the starting distribution is \( a_0 \). To ensure the proportion of the Gaussian distribution with \( x < 0 \) is insignificant \( \theta_0^2 \) is chosen to be very small. It follows from Equations (2.29)-(2.32) that the initial number of cells is given by

\[
\int_0^\infty G_1(x, 0)dx = \frac{a_0}{2} \left(1 + \text{erf}\left(\frac{1}{\sqrt{2}\theta_0}\right)\right). \quad (2.33)
\]

An additional boundary condition is required to ensure cells always have a positive DNA content, i.e. the dispersion multiplied by the change in DNA is equal to the growth in DNA for cells with zero DNA content. This is represented mathematically as

\[
D\frac{\partial S}{\partial x}(0, t) - gS(0, t) = 0, \quad t > 0. \quad (2.34)
\]

Equations (2.22)-(2.34) fully describe the system. A graphical representation of the model is shown in Figure 2.1.
In [61] it is shown that for a population with all phases of infinite length and with constant transition rates that once the population has attained a steady DNA distribution (i.e. for a population which is undergoing exponential growth) the average time cells spend in each phase is linked to the transition rate parameters by the formulae

\[ T_{G_1} = \frac{1}{k_1 + \mu_{G_1}}, \]  

(2.35)
2.2 Partial Differential Equation Models

\[ T_S = \frac{1}{g + \mu_S}, \]  
\[ T_{G_2} = \frac{1}{k_2 + \mu_{G_2}}, \]  
\[ T_M = \frac{1}{b + \mu_M}. \]  

These relationships only hold for when all phases are of infinite length with a constant transition rate. For the model described by Equations (2.22)-(2.34) this is not the case, as the \( S \) phase is of fixed duration. Both Equations (2.35)-(2.38) and Steel’s formulae, detailed in Section 2.1.1, are used in [3]. Basse et al. estimated the time cells spent in each phase by measuring the percentage of cells in each phase experimentally then using Steel’s formulae. The values obtained for the time the cells spent in each phase were then used in Equations (2.35)-(2.38) to obtain an estimate for the parameters \( k_1, g, k_2 \) and \( b \).

There appears to be some discrepancy between the use of these two sets of equations as the values for the time spent in each phase that are used to obtain parameter estimates from Equations (2.35)-(2.38) do not agree with the values for the time spent in each phase obtained when the cell phase distribution is placed into Steel’s formulae. The source for this discrepancy is the use of Steel’s formulae with a fixed duration \( S \) phase; this affects the \( k_2 \) parameter.

**Age Structured Models**

As discussed in Section 2.2.1 a single compartment, age-structured model of a population of cells was considered in [38] using a successive generations approach. The formulation of the model and solutions are similar to those previous discussed in Section 2.2.1 for the mass structured approach. Further analysis of this type of model and the form of the solutions which may occur may be found in [17] where overcrowding and mortality are considered. Once again periodic and steady state solutions were found.

Both a healthy population of cells and a population of cells exposed to a variety of cancer treatments, in particular the chemotherapy drug pacitaxel, was modelled using an age structured population balance framework in [6]. The approach for obtaining a solution to the equations for the model is similar to that discussed in Chapter 4.
In [6] it is noted that the most convenient way of validating the model and the resulting cell populations was to compare the model with experimental DNA profiles obtained using flow cytometry. In order to do this DNA profiles must be inferred from the information contained in the model regarding the ages of cells within the population. This was done by assuming the DNA content of cells in the $P$ phase where $P \in \{G_1, G_2, M\}$ fits a Gaussian distribution given by

$$
\mathcal{F}_P(x, t) = \frac{N_P(t)}{\sqrt{2\pi\theta^2_P}} \exp\left(\frac{-(x - \mu_P)^2}{2\theta^2_P}\right),
$$

where $x$ is the relative DNA content, $\mu_P$ is the mean DNA content for a cell in the $P$ phase and $\theta^2_P$ is the variance for the distribution. For the $S$ phase the DNA profile is given by

$$
\mathcal{F}_S(x, t) = \int_0^\infty \frac{N_S(t, \tau_S)}{\sqrt{2\pi\theta^2_S(\tau_S)}} \exp\left(\frac{-(x - \mu_S(\tau_S))^2}{2\theta^2_S(\tau_S)}\right) d\tau_S,
$$

It should be noted that for the $S$ phase the mean and variance of the population are age dependent.

Whilst this work provides a good general framework for the solution of a multi compartment, age structured model it is assumed that the rates that cells transition between the phases is constant and independent of their age. Although this simplifies the problem it has not been examined in depth. Since the transition between the $G_1$ and $S$ phases is independent of age, cells are assumed to be viable for entering the $S$ phase immediately upon starting the $G_1$ phase, as mentioned in Section 2.2.1, this is biologically unrealistic. These issues are addressed in the remainder of Section 2.2.1.

**CelCyMUS Age Structured Model**

In [22], [23] and [57] an age structured model, known as CelCyMUS, developed at the University of Surrey is discussed. This model consists of six compartments, the $G_1$ phase is divided into two parts $G_{1a}$ and $G_{1b}$, the other compartments being the $S$, $G_2$ and $M$ phases and an additional $G'$ phase. The initial $G'$ phase is introduced so that the model may accurately represent *in-vitro* experiments where the cell population has been partially synchronised with a chemical block. It is from this phase cells initially enter the cell cycle. The $G_1$ phase is subdivided into two parts $G_{1a}$ and $G_{1b}$. This is done to address the issue of cells being able to transition from the $G_1$ to $S$ phase before they have
had time to grow and acquire the minimum amount of nutrients required for successful completion of the cycle. The $G_{1b}$ compartment is of a fixed time duration, representing the minimum amount of time a cell must be in the $G_1$ phase before being able to progress to the $S$ phase. During $G_{1b}$ cells ‘transition’ to the $S$ phase via a probability distribution function which may be dependent on age, nutrient levels or other growth limiting factors. This ‘transition’ of cells between $G_{1b}$ and the $S$ phase is discussed in detail in Section 3.3. Cells who have not left $G_{1b}$ after a certain time are assumed to enter a death compartment $D$ where they are no longer viable. Once in the death compartment $D$, the cells leave (i.e. break down) at a rate which is related to the concentration of ammonia in the media; the ammonia is a by-product of the viable cells consuming glutamine. The death compartment is not discussed further but is merely described here for completeness.

Age is a natural choice for the independent variable to use for the population balance framework, as this allows the model to incorporate the age dependent nature of the transition from the $G_{1b}$ phase to the $S$ phase. A graphical representation of the CelCyMUS model is shown in Figure 2.2. In addition to the equations governing the cell populations in the various phases the model also considers the concentration of growth limiting nutrients. In [23] the model is validated using experimental data presented in [31] and concerns a batch experiment which was conducted using a mouse-mouse hybridoma cell line (nm321). In this model glutamine is considered as the sole growth limiting nutrient, however this may easily be extended to include other growth limiting factors.
Equations governing the model

Since the CelCyMUS model is used as motivation for the construction of a new simplified model in Section 3.2, a full derivation of the equations governing the CelCyMUS model is necessary.

Nutrient Equation

If the glutamine concentrations in the media surrounding the cells, the flow stream of media into the region of interest and the glutamine within a cell are denoted by $C_{GLUT}$, $C_{QGLUT}$ and $C_{CGLUT}$ respectively, then
\[
\frac{dC_{\text{GLUT}}(t)}{dt} = \left( C_{Q_{\text{GLUT}}}(t) - C_{\text{GLUT}}(t) \right) \frac{Q(t)}{V(t)} - R_{\text{GLUT}} \left( \int_0^{T_{G_{1a}}} n_{G_{1a}}(t, \tau_{G_{1a}})d\tau_{G_{1a}} + \int_0^{T_{G_{1b}}} n_{G_{1b}}(t, \tau_{G_{1b}})d\tau_{G_{1b}} \right), \quad (2.41)
\]

where \( Q(t) \) is the flow rate of the media into the region of interest, \( V(t) \) is the volume of the media in the region of interest and \( \frac{R_{\text{GLUT}}}{2} \) is the rate that cells in both \( G_{1a} \) and \( G_{1b} \) absorb glutamine i.e.

\[
\frac{R_{\text{GLUT}}}{2} = \frac{\partial C_{\text{CGGLUT}}(t, \tau_{G_{1b}})}{\partial t} = \frac{\partial C_{\text{CGGLUT}}(t, \tau_{G_{1a}})}{\partial t}. \quad (2.42)
\]

It should be noted that the rate cells absorb glutamine is given by \( \frac{R_{\text{GLUT}}}{2} \) so that full derivative along the characteristics \( t = \tau + \text{constant} \) is given by \( R_{\text{GLUT}} \). Furthermore, \( R_{\text{GLUT}} \) is assumed to be a Heaviside function of \( C_{\text{GLUT}} \) such that \( R_{\text{GLUT}} \) is constant if \( C_{\text{GLUT}} > 0 \) and zero otherwise.

Since [23] uses experimental batch data \( Q(t) = 0 \), thus Equation (2.41) simplifies to

\[
\frac{dC_{\text{GLUT}}(t)}{dt} = -R_{\text{GLUT}} \left( \int_0^{T_{G_{1a}}} n_{G_{1a}}(t, \tau_{G_{1a}})d\tau_{G_{1a}} + \int_0^{T_{G_{1b}}} n_{G_{1b}}(t, \tau_{G_{1b}})d\tau_{G_{1b}} \right). \quad (2.43)
\]

**\( G_{1a} \) Phase Equation**

To derive the population balance equation for the cells in the \( G_{1a} \) portion of the \( G_1 \) phase it is useful to first describe the parts of the equation in words. Thus,

\[
\begin{align*}
\text{Change in number of cells,} & \quad \text{Cells flowing out (washout)} \\
\text{whose age } & \in \left[ \tau, \tau + \delta \tau \right), \quad + \\
\text{in the time interval } & \left[ t, t + \delta t \right) \quad \text{Cells leaving due to ageing} \\
& \quad + \\
& \quad \text{Cells joining due to ageing} \\
& \quad + \\
& \quad \text{Cells leaving or joining due to transition rules.} \quad (2.44)
\end{align*}
\]
2.2 Partial Differential Equation Models

Each of the parts of this equation are now considered in turn.

The number of the cells is given by the volume of the system \( V(t) \) multiplied by the cell density. The change in the number of cells in the time interval \( [t, t + \delta t] \) is the difference between the number at time \( t \) and time \( t + \delta t \). Hence, the left hand side of Equation (2.44) may be expressed as

\[
\text{Change in number of cells}
\]

whose age \( \in [\tau, \tau + \delta \tau] \)

\[
\int_{\tau}^{\tau + \delta \tau} (V(t + \delta t)n_{G_{1a}}(t + \delta t, \sigma) - V(t)n_{G_{1a}}(t, \sigma)) \, d\sigma,
\]

in the time interval \( [t, t + \delta t] \)

(2.45)

where \( n_{G_{1a}}(t, \tau) \) is the cell density in the \( G_{1a} \) phase. Using integration by parts and the product rule, the integrand in Equation (2.45) may itself be written in terms of an integral as

\[
(V(t + \delta t)n_{G_{1a}}(t + \delta t, \sigma) - V(t)n_{G_{1a}}(t, \sigma)) = \int_t^{t+\delta t} \frac{\partial}{\partial s}(V(s)n_{G_{1a}}(s, \sigma)) \, ds
\]

\[
= \int_t^{t+\delta t} \frac{dV(s)}{ds}n_{G_{1a}}(s, \sigma) + V(s)\frac{\partial}{\partial s}(n_{G_{1a}}(s, \sigma)) \, ds.
\]

Hence, Equation (2.45) may be rewritten as

\[
\text{Change in number of cells}
\]

whose age \( \in [\tau, \tau + \delta \tau] \)

\[
\int_{t}^{\tau + \delta \tau + t} \int_{\tau}^{\tau + \delta \tau} \frac{dV(s)}{ds}n_{G_{1a}}(s, \sigma) + V(s)\frac{\partial}{\partial s}(n_{G_{1a}}(s, \sigma)) \, d\sigma ds.
\]

in the time interval \( [t, t + \delta t] \)

(2.47)

It is assumed that there is no flow of cells into the region of interest so the only flow is cells leaving due to washout. Washout from the region of interest may represent cells leaving in the blood stream \textit{in-vivo} or from an exit stream of an \textit{in-vitro} continuous stirred-tank reactor (CSTR) experiment. The change in the number of cells whose age is \( \in [\tau, \tau + \delta \tau] \) due to washout is the flow rate out \( Q_{out}(t) \) multiplied by the change in cell density over the time interval \( [t, t + \delta t] \), summed over the biological age period of interest. Thus, for cells whose age is \( \in [\tau, \tau + \delta \tau] \) the term related to cell washout may be written as

\[
- \int_t^{t+\delta t} \int_{\tau}^{\tau + \delta \tau} Q_{out}(s)n_{G_{1a}}(s, \sigma) \, d\sigma ds.
\]

(2.48)
The second term on the right hand side of Equation (2.44) represents cells who leave due to ageing; their total number is given by the volume of the system $V(t)$ multiplied by the cell density summed over the time interval $[t, t + \delta t]$. As we are only concerned with the oldest cells in the age interval $[\tau, \tau + \delta \tau)$ leaving there is no summation over $\tau$ so this term can be expressed as

$$- \int_{t}^{t+\delta t} V(s)n_{G_{1a}}(s, \tau + \delta \tau) \, ds. \quad (2.49)$$

In the same manner, cells entering the age interval $[\tau, \tau + \delta \tau)$ due to ageing may be written as

$$\int_{t}^{t+\delta t} V(s)n_{G_{1a}}(s, \tau) \, ds. \quad (2.50)$$

Using both integration by parts and the product rule the sum of Equations (2.49) and (2.50) may now be written as

$$- \int_{t}^{t+\delta t} \int_{\tau}^{\tau+\delta \tau} V(s) \frac{\partial}{\partial \sigma} n_{G_{1a}}(s, \sigma) \, d\sigma ds. \quad (2.51)$$

The final term in Equation (2.44) concerns the cells that leave or join due to the transition rates $R_{G_{1a}}$, which depends on the number of cells available for transitioning at a given time and may also depend on a number of different variables, including cell age $\tau$, nutrient concentrations and time. The change in number of cells is given by the volume of cells $V(t)$ multiplied by the transition rate (units are number per volume per time i.e. cell density per time), summed over the biological age and time intervals of interest. Thus for cells of age $\in [\tau, \tau + \delta \tau)$ over the time interval $[t, t + \delta t)$ the number of cells leaving due to transition is given by

$$- \int_{t}^{t+\delta t} \int_{\tau}^{\tau+\delta \tau} V(s)R_{G_{1a}}(x) \, d\sigma ds, \quad (2.52)$$

where $x$ denotes the variables upon which the transition rate depends.

Using Equations (2.47), (2.48), (2.51), and (2.52), Equation (2.44) may now be rewritten as

$$\int_{t}^{t+\delta t} \int_{\tau}^{\tau+\delta \tau} \frac{dV(s)}{ds} n_{G_{1a}}(s, \sigma) + V(s) \frac{\partial}{\partial \sigma} \{n_{G_{1a}}(s, \sigma)\} \, d\sigma ds =$$

$$- \int_{t}^{t+\delta t} \int_{\tau}^{\tau+\delta \tau} Q_{out}(s)n_{G_{1a}}(s, \sigma) + V(s) \frac{\partial}{\partial \sigma} n_{G_{1a}}(s, \sigma) + V(s)R(n_{G_{1a}}) \, d\sigma ds. \quad (2.53)$$
The change in the volume of the system at time $t$ is given by the flow rate in $Q(t)$ minus the flow rate out $-Q_{out}(t)$. Thus, the flow rate into the region of interest $Q(t)$ is given by

$$Q(t) = \frac{dV(t)}{dt} + Q_{out}(t).$$

Using this Equation (2.53) may be simplified to

$$\int_{t}^{t+\delta t} \int_{\tau}^{\tau+\delta \tau} V(s) \left\{ \frac{\partial}{\partial s} n_{G_{1a}}(s, \sigma) - \frac{Q(s)}{V(s)} n_{G_{1a}}(s, \sigma) + \frac{\partial}{\partial \sigma} n_{G_{1a}}(s, \sigma) + R(x) \right\} \, d\sigma ds = 0.$$

Since $V(s) > 0 \, \forall s \in \mathbb{R}, \forall t$ it follows that

$$\left\{ \frac{\partial}{\partial s} n_{G_{1a}}(s, \sigma) - \frac{Q(s)}{V(s)} n_{G_{1a}}(s, \sigma) + \frac{\partial}{\partial \sigma} n_{G_{1a}}(s, \sigma) + R(x) \right\} = 0,$$

i.e.

$$\left\{ \frac{\partial}{\partial t} n_{G_{1a}}(t, \tau_{G_{1a}}) - \frac{Q(t)}{V(t)} n_{G_{1a}}(t, \tau_{G_{1a}}) + \frac{\partial}{\partial \tau_{G_{1a}}} n_{G_{1a}}(t, \tau_{G_{1a}}) + R(x) \right\} = 0.$$

This is the population balance equation presented in [23] where

$$R_{1G_{1a}}(n_{G_{1a}}(t, \tau_{G_{1a}}), \tau_{G_{1a}}) = \delta(\tau_{G_{1a}} - T_{G_{1a}}) n_{G_{1a}}(t, \tau_{G_{1a}}),$$

is the transition rate defined such that cells cannot leave the $G_{1a}$ part of the $G_1$ phase until they have reached a certain age.

**$G_{1a}$ Phase Boundary Conditions**

To account for new cells entering the $G_1$ phase the following boundary condition is required

$$n_{G_{1a}}(t, 0) = \begin{cases} 2n_M(t, T_M) & \text{for } C_{GLUT}(t) > 0, \\ 0 & \text{for } C_{GLUT}(t) = 0, \end{cases}$$

where $T_M$ is the maximum age of cells in the $M$ phase of the cycle. It should be noted that cells at the end of the $M$ phase are only able to divide and produce daughter cells if there are nutrients available, otherwise no daughter cells are produced. This discontinuous boundary condition may be modified to account for limited nutrients.

The derivation of the equations governing cells in the other phases follows in a similar fashion to the derivation of the equations for the $G_{1a}$ phase. This leads to the following set of equations.
2.2 Partial Differential Equation Models

\( G_{1b} \) Phase Equation

Let \( n_{G_{1b}}(t, \tau_{G_{1b}}) \) denote the density of cells of age \( \tau_{G_{1b}} \) in the \( G_{1b} \) phase at time \( t \) then

\[
\frac{\partial}{\partial t} n_{G_{1b}}(t, \tau_{G_{1b}}) - \frac{Q(t)}{V(t)} n_{G_{1b}}(t, \tau_{G_{1b}}) + \frac{\partial}{\partial \tau_{G_{1b}}} n_{G_{1b}}(t, \tau_{G_{1b}}) \\
+ R_{1G_{1b}}(n_{G_{1b}}(t, \tau_{G_{1b}}), \tau_{G_{1b}}) + R_{2G_{1b}}(n_{G_{1b}}(t, \tau_{G_{1b}}), \tau_{G_{1b}}) = 0,
\]

(2.59)

where

\[
R_{1G_{1b}}(n_{G_{1b}}(t, \tau_{G_{1b}}), \tau_{G_{1b}}) = \delta(\tau_{G_{1b}} - T_{G_{1b}}) n_{G_{1b}}(t, \tau_{G_{1b}})
\]

(2.60)

and

\[
R_{2G_{1b}}(n_{G_{1b}}(t, \tau_{G_{1b}}), \tau_{G_{1b}}) = \frac{2n_{G_{1b}}(t, \tau_{G_{1b}})}{S_{MAX} - C_{CGLUT}(t, \tau_{G_{1b}})} \frac{\partial C_{CGLUT}(t, \tau_{G_{1b}})}{\partial t},
\]

(2.61)

where \( S_{MAX} \) represents the total amount of glutamine a cell may consume before it is forced to leave the \( G_{1b} \) phase and enter the \( S \) phase.

\( R_{2G_{1b}}(n_{G_{1b}}(t, \tau_{G_{1b}}), \tau_{G_{1b}}) \) represents the transition from the \( G_{1b} \) to the \( S \) phase. This transition function is used in [23] however it may be replaced with any suitable transition function as discussed in Section 3.3.

\( G_{1b} \) Phase Boundary Conditions

\[
n_{G_{1b}}(t, 0) = n_{G_{1b}}(t, T_{G_{1b}}) + n_{GV}(t, T_{GV}).
\]

(2.62)

\( S \) Phase Equation

Let \( n_{S}(t, \tau_{S}) \) denote the density of cells of age \( \tau_{S} \) in the \( S \) phase at time \( t \) then

\[
\frac{\partial}{\partial t} n_{S}(t, \tau_{S}) - \frac{Q(t)}{V(t)} n_{S}(t, \tau_{S}) + \frac{\partial}{\partial \tau_{S}} n_{S}(t, \tau_{S}) + R_{1S}(n_{S}(t, \tau_{S}), \tau_{S}) = 0,
\]

(2.63)

where

\[
R_{1S}(n_{S}(t, \tau_{S}), \tau_{S}) = \delta(\tau_{S} - T_{S}) n_{S}(t, \tau_{S})
\]

(2.64)
2.2 Partial Differential Equation Models

**S Phase Boundary Conditions**

The number of cells entering the S phase at time \( t \) is given by the total number of cells, of all ages, who leave the \( G_{1b} \) phase via the \( R_{2G_{1b}} \) transition function, defined as

\[
n_S(t,0) = \int_{0}^{\tau_{G_{1b}}} \frac{2n_{G_{1b}}(t,\tau_{G_{1b}})}{S_{MAX} - C_{GGLUT}(t,\tau_{G_{1b}})} \frac{\partial C_{GGLUT}(t,\tau_{G_{1b}})}{\partial t} d\tau_{G_{1b}}. \tag{2.65}
\]

**\( G_2 \) Phase Equation**

Let \( n_{G_2}(t,\tau_{G_2}) \) denote the density of cells of age \( \tau_{G_2} \) in the \( G_2 \) phase at time \( t \) then

\[
\frac{\partial}{\partial t} n_{G_2}(t,\tau_{G_2}) - \frac{Q(t)}{V(t)} n_{G_2}(t,\tau_{G_2}) + \frac{\partial}{\partial \tau_{G_2}} n_{G_2}(t,\tau_{G_2}) + R_{1G_2}(n_{G_2}(t,\tau_{G_2}),\tau_{G_2}) = 0, \tag{2.66}
\]

where

\[
R_{1G_2}(n_{G_2}(t,\tau_{G_2}),\tau_{G_2}) = \delta(\tau_{G_2} - T_{G_2}) n_{G_2}(t,\tau_{G_2}) \tag{2.67}
\]

**\( G_2 \) Phase Boundary Conditions**

\[
n_{G_2}(t,0) = n_S(t,T_S). \tag{2.68}
\]

**\( M \) Phase Equation**

Let \( n_M(t,\tau_M) \) denote the density of cells of age \( \tau_M \) in the \( M \) phase at time \( t \) then

\[
\frac{\partial}{\partial t} n_M(t,\tau_M) - \frac{Q(t)}{V(t)} n_M(t,\tau_M) + \frac{\partial}{\partial \tau_M} n_M(t,\tau_M) + R_{1M}(n_M(t,\tau_M),\tau_M) = 0, \tag{2.69}
\]

where

\[
R_{1M}(n_M(t,\tau_M),\tau_M) = \delta(\tau_M - T_M) n_M(t,\tau_M) \tag{2.70}
\]

**\( M \) Phase Boundary Conditions**

\[
n_M(t,0) = n_{G_2}(t,T_{G_2}). \tag{2.71}
\]

**\( G' \) Phase**

The \( G' \) phase represents an initial quiescent phase which may occur in an *in-vitro* setting where some form of cell blocking technique has been used. There are no equations for this
phase as cells are only in this phase at the start so this may be thought of as an ‘initial’ phase with the initial cells of varying age. Therefore at \( t = 0 \), \( 0 \leq \tau_{0} < T_{0} \).

The model outlined by Equations (2.41-2.71) form the basis of the CelCyMUS model presented in [23] and [22].

### 2.3 Summary of Existing Cell Cycle Models

In this chapter a number of existing cell cycle models have been considered. Some of the approaches utilising ODEs to model a population of cells were discussed in Section 2.1. Using Steel’s formulae, which were derived in Section 2.1.1, it was shown that it is possible to obtain the proportion of cells in each of the phases from a single unstructured ODE model. The idea of structured ODE models was also introduced and a number of existing structured ODE models briefly discussed. In Section 2.1.3 some Molecular ODE models were reviewed. Whilst appreciating the importance of these models, this type of model was not considered further as it is not possible to gain much insight into the model from studying the complex system of governing equations.

Whilst it is possible to construct a model which provides a good representation of the cell population as a whole using ODEs this approach does not consider the intraphase heterogeneity of the population. For this it is necessary to model the population using a PDE model. There are a number of different approaches for modelling cell population dynamics such as Markov chains [65], but the most common approach is to use partial differential equations, to take into account the continuous variability of the cell properties. A comprehensive review of existing population balance models was given in Section 2.2.1.

Section 2.2 began with a brief introduction of the population balance framework, as detailed in [20], [25] and [24], together with an overview of the most common types of structuring used for a population of cells. The concept of partition functions for mass and DNA structured population balance models was introduced and the advantage of not needing a partition function for an age structured model was also discussed. DNA, mass and multi-variable models were reviewed with both a single, generation type approach and a multi-cell phase approach being considered.

A detailed review of the DNA structured framework presented by Basse et al. in [3] was undertaken. Whilst such models may easily be verified by direct comparison with
observed DNA content there are also a number of shortcomings to the model given in [3], namely

- The observational error in measuring relative DNA content caused by differing orientations of the nucleus when passing through the flow cytometer is included directly into the model. Whilst this is important this should be applied as an additional transformation to the data at the end of the model.

- Cells are assumed to be viable for entering the $S$ phase immediately upon starting the $G_1$ phase, this is unrealistic as there is a minimum time cells must remain in $G_1$, as certain specific processes such as mass accumulation and protein synthesis must be at least partially completed, before progressing further round the cycle [45]. This issue could be addressed by dividing the $G_1$ phase into two parts, one of which, like the $S$ phase, would be of fixed duration. This would ensure cells remain in the $G_1$ phase for a minimum period of time before progressing through the cycle.

- Even if cell death during the cycle is assumed to be zero (i.e. $\forall i \in \{G_1, S, G_2, M\}, \mu = 0$) there are still a number of parameters ($k_1, g, k_2, \theta, b$ and $a_0$) which need to be fitted. Although estimates on most of these can be obtained from experimental data the parameters still need to be optimised for best fit.

In Section 2.2.1 age structured PDEs are considered. The structure used in [6] was discussed and whilst this work provides a good general framework for the solution of a multi compartment, age structured model it is assumed that the rates that the cells transition between the phases is constant and independent of their age. Because the transition between the $G_1$ and $S$ phases in this model is independent of age, cells were assumed to be viable for entering the $S$ phase immediately upon starting the $G_1$ phase, this is biologically unrealistic. These issues are addressed by the CelCyMUS model given by [22], [23] and [57]. In Section 2.2.1 the age structured PDE model, CelCyMUS, was reviewed in depth. This work consists of a detailed and accurate model, which was verified against experimental data, and has since been extended to consider external factors such as radiation [35]. Despite this model providing a good fit to experimental data there are issues concerning the validity of the transition functions used in [23] and [57], this is discussed further in Section 3.3. A number of simplifications may be made without
compromising the model’s ability to accurately simulate and fit experimental data. These simplifications and a new simplified age structured population balance model are presented in Chapter 3.

In conclusion, it is possible to obtain some information on a population of cells using a structured ODE model. However, if the heterogeneity of the population is to be accounted for then a PDE model must be used. Most PDE models have a population balance framework with a variety of variables being used to structure the model. By using an age structured model it is possible to control the length of time that cells may remain in the cell cycle and infer the mass and DNA distributions. Since there is not a one-to-one mapping between age and DNA or mass the converse is not true. Age structured models have the additional benefit that there is no need for a partition function. As detailed in Section 2.2.1 there are a number of existing age structured PDE models, but these models have their limitations and may be unnecessarily over complicated.
Chapter 3

Validation and Comparison of New Ordinary Differential Equation and Partial Differential Equation Models

In this Chapter new ODE and PDE models are formulated. The new ODE model gives an alternative method for calculating the percentage of cells in each phase from Steel’s formulae. Whilst Steel’s formulae infers information from the relative phase lengths the new ODE model allows the percentage of cells in each phase to be obtained directly from the ODE model. The ODE model presented also allows for a good comparison of ODE and PDE models and forms a general framework which is extended in Chapter 6. The PDE model presented is compared against the CelCyMUS model ([22]; [23] and [57]) upon which it is based and, once verified, the new PDE model is compared against the ODE modelling approach.

In Section 3.1 the new structured ODE model is constructed. This model consists of four compartments, each corresponding to a different phase of the cell cycle. The rate constants, which define the rates at which cells pass between these compartments are chosen to all be unity allowing an analytic solution to be obtained. The model is then used to predict the phase distribution of the population and these results are then compared to Steel’s formulae.

Section 3.2 starts with a brief discussion of simplifications which may be made to the
3.1 Structured Ordinary Differential Equation Model

CelCyMUS model, discussed in Section 2.2.1. Using the CelCyMUS model as a starting point a new three compartment, age-structured PDE model is presented. The review given in Chapter 2 highlights the fact that cells only progress from the $G_1$ to $S$ phase of the cycle after they have grown sufficiently and taken on enough nutrients to complete the cycle. In Section 3.3 two different approaches for deriving the transition function which describes how cells transition from the $G_1$ phase to $S$ phase are detailed. A constant transition function of the form used in [6] is then briefly discussed. The quadratic and sigmoidal functions used in [22], [23] and [57] are then discussed in detail, with respective flaws highlighted. A new, sigmoidal transition function is then presented together with an appropriate non-dimensionalisation consistent with the non-dimensionalisation given in Section 3.2 for the new age-structured model.

In Section 3.4 a numerical scheme for the new age-structured model is presented, together with a discussion on the scheme’s stability. Section 3.5 sees this new age-structured model with different transition functions compared with experimental data. The experimental data concerns a batch experiment which was conducted using a mouse-mouse hybridoma cell line (mm321) [31].

In Section 3.6 the ODE model framework discussed in Section 3.1 is modified to allow direct comparison with the PDE model described in Section 3.2. The ODE model is then fitted to the same experimental data as that used in Section 3.5 and the ODE and PDE results are compared.

The chapter concludes with a brief summary of the findings.

3.1 Structured Ordinary Differential Equation Model

By considering the different phases within the cell cycle it is possible to create a simple, structured model which includes some information on the cells’ progression through the cell cycle. In this Section an approach is used where there are a number of compartments each containing a portion of the cell cycle, i.e. four compartments each containing one of the four phases, $G_1$, $S$, $G_2$ and $M$. This type of model provides a better description on the population of cells being modelled than those of [54] and [62] discussed in Section 2.1.2, and is of a similar form to the model presented in [52]. This type of compartmental model has been chosen as it may be used to obtain qualitative information about the
growth kinetics of the population. The model that is detailed in this section is similar to that discussed in [52], in as much as each compartment represents a phase of the cell cycle however the quiescent $G_0$ phase is omitted. As the population of cells which are to be modelled excludes any quiescent $G_0$ phase the population is assumed to be closed, i.e. no net flow of cells into or out of the population. The population may be represented as four compartments each of which contain one phase, as shown in Figure 3.1, where $k_i, i \in \{1, 2, 3, 4\}$ represents the rate cells pass between the different phases.

![Cell Cycle Diagram](Image)

Figure 3.1: Compartmental Representation of the Cell Cycle.

Unlike the model detailed in [52], there is no regulatory molecule included as the $G_0$ phase has been omitted, this simplifies the resulting ODEs governing the population kinetics.
Furthermore, no cell death is included at this stage, however this is considered in Chapter 6, where the non linear effects of chemotherapy drugs are included. 

If the rate constants \( k_i \) are assumed to be constant then this compartment model may be represented mathematically by four linear first order ODEs, one for each compartment:

\[
\frac{dN_{G_1}(t)}{dt} = 2k_1 N_M(t) - k_2 N_{G_1}(t), \quad (3.1a)
\]

\[
\frac{dN_S(t)}{dt} = k_2 N_{G_1}(t) - k_3 N_S(t), \quad (3.1b)
\]

\[
\frac{dN_{G_2}(t)}{dt} = k_3 N_S(t) - k_4 N_{G_2}(t), \quad (3.1c)
\]

\[
\frac{dN_M(t)}{dt} = k_4 N_{G_2}(t) - k_1 N_M(t), \quad (3.1d)
\]

where \( N_{G_1}(t), N_S(t), N_{G_2}(t) \) and \( N_M(t) \) are the number of cells in the four compartments at time \( t \). Equations (3.1) may be solved analytically to give:

\[
N_{G_1}(t) = \sum_{j=1}^{4} C_j e^{R_j t},
\]

\[
N_S(t) = \frac{1}{2k_1 k_3 k_4} \left( \sum_{j=1}^{4} C_j R_j^3 e^{R_j t} \right) + \frac{k_1 + k_2 + k_4}{2k_1 k_3 k_4} \left( \sum_{j=1}^{4} C_j R_j^2 e^{R_j t} \right) + \frac{k_1 k_2 k_3}{2k_1 k_3 k_4} \left( \sum_{j=1}^{4} C_j R_j e^{R_j t} \right),
\]

\[
N_{G_2}(t) = \frac{1}{2k_1 k_4} \left( \sum_{j=1}^{4} C_j R_j^2 e^{R_j t} \right) + \frac{k_1 + k_2}{2k_1 k_4} \sum_{j=1}^{4} C_j e^{R_j t} + \frac{k_2}{2k_4} \sum_{j=1}^{4} C_j e^{R_j t},
\]

\[
N_M(t) = \frac{1}{2k_1} \sum_{j=1}^{4} C_j (R_j + k_1)(R_j + k_2) e^{R_j t},
\]
where \( R_j, j \in \{1, 2, 3, 4\} \) are the roots of the characteristic polynomial

\[
R^4 + \left( \sum_{i=1}^{4} k_i \right) R^3 + \left( \sum_{i=1}^{3} \sum_{j=i+1}^{4} k_i k_j \right) R^2 + \left( \sum_{i=1}^{2} \sum_{j=i+1}^{3} \sum_{m=j+1}^{4} k_i k_j k_m \right) R - \prod_{i=1}^{4} k_i = 0, \tag{3.3}
\]

and \( C_j, j \in \{1, 2, 3, 4\} \) are constants which may be determined from the initial conditions. Note Equation (3.3) may be rewritten as

\[
\prod_{i=1}^{4} (R + k_i) = 2 \prod_{i=1}^{4} k_i, \tag{3.4}
\]

this result is required later in the simplification of Equation (3.8). From Equations (3.2) for \( t \gg 1 \)

\[
N_{G_1}(t) \approx C^* e^{R^* t},
\]

\[
N_S(t) \approx \frac{1}{2k_1k_3k_4} \left( C^* (R^* + k_1)(R^* + k_2)(R^* + k_4) e^{R^* t} \right),
\]

\[
N_{G_2}(t) \approx \frac{1}{2k_1k_3} C^* (R^* + k_1)(R^* + k_2) e^{R^* t},
\]

\[
N_M(t) \approx \frac{1}{2k_1} C^* (R^* + k_2) e^{R^* t},
\]

where \( R^* = \max \{ \Re(R_i) \mid i \in \{1, 2, 3, 4\} \} \) and \( C^* \) is the corresponding constant. For the special case where there is a pair of complex conjugate roots with largest real part then \( C^* = \sum C_j \forall j \) such that \( R_j = \max \{ \Re(R_i) \mid i \in \{1, 2, 3, 4\} \} \). Should repeated roots \( R_a = R_b \) occur then each \( C_b \) is replaced with \( C_b t \) in Equations (3.2). If in this case \( R_a = R_b = R^* \) then all of the expressions on the right hand side of Equations (3.5) are multiplied by \( t \).

The fraction of the total population in phase \( q \in \{G_1, S, G_2, M\} \) at time \( T \) is given by

\[
P_q(T) = \frac{N_q(T)}{N_{G_1}(T) + N_S(T) + N_{G_2}(T) + N_M(T)} \quad q \in \{G_1, S, G_2, M\}. \tag{3.6}
\]

Thus, using Equations (3.5) and (3.6), for sufficiently large \( t \) the fraction of the population in the \( G_1 \) phase at time \( t \) is estimated by

\[
P_{G_1}(T) = \frac{C^* e^{R^* t}}{\Theta}, \tag{3.7}
\]
where
\[ \Theta = C^* e^{R^* T} + \frac{1}{2k_1k_3k_4} \left( C^* (R^* + k_1)(R^* + k_2)(R^* + k_3)e^{R^* T} \right) \]
\[ + \frac{1}{2k_1k_3} C^* (R^* + k_1)(R^* + k_2)e^{R^* T} + \frac{1}{2k_1} C^* (R^* + k_2)e^{R^* T}. \]

Equation (3.7) may be simplified to give
\[ P_{G_1}(T) = \frac{2k_1k_3k_4}{(R^* + k_1)(R^* + k_2)(R^* + k_3) + (R^* + k_1)(R^* + k_2)(R^* + k_3) + (R^* + k_2)(R^* + k_3) + 2k_1k_3k_4}. \]

By using Equation (3.4) it is possible to rearrange Equation (3.8) to give
\[ P_{G_1}(T) = \frac{(R^* + k_1)(R^* + k_3)(R^* + k_4)}{(R^* + k_1)(R^* + k_3)(R^* + k_4) + (R^* + k_1)(R^* + k_4)(R^* + k_2) + (R^* + k_2)(R^* + k_3) + 2k_1k_3k_4}. \]

it is this form that will be used for comparison with the results obtained using Steel’s formulae.

Similar expressions may be obtained for the other phases, $S$, $G_2$ and $M$.

### 3.1.1 Comparison of Results Obtained Using Steel’s Formulae and the Four Compartment ODE Model

Consider the special case where $k_i = 1, i \in \{1, 2, 3, 4\}$ and the initial conditions given by
\[ N_{G_1}(0) = N_0, \quad (3.10a) \]
\[ N_S(0) = 0, \quad (3.10b) \]
\[ N_{G_2}(0) = 0, \quad (3.10c) \]
\[ N_M(0) = 0. \quad (3.10d) \]

As previously discussed in Section 2.2.1 these initial conditions may arise when modelling an *in-vitro* population of cells which has been subject to some form of synchronisation which results in all the cells in the population being in the same phase. Further details of this type of blocking process may be found in [23] and [31].
The characteristic polynomial given by Equation (3.4) is now

\[(R + 1)^4 = 2.\]  

(3.11)

The roots of Equation (3.11) are

\[R_j = i^j 2^{j/2} - 1, \quad j \in \{1, 2, 3, 4\}.\]

(3.12)

Let \(R^* = \max \{\Re(R_i) \mid i \in \{1, 2, 3, 4\}\} = 2^{1/2} - 1\). Using this value of \(R^*\) and \(k_i = 1, i \in \{1, 2, 3, 4\}\) in Equation (3.9) gives

\[P_{G_1}(t) = \frac{2^{1/2}}{2^{3/2} + 2^{1/2} + 2^{1/2} + 1},\]

\[= \frac{2^{1/2}}{\left(2^{1/2} + 1\right)\left(2^{1/2} + 1\right)},\]

\[= \frac{2^{1/2}(2^{1/2} - 1)}{(2^{1/2} + 1)(2^{1/2} + 1)(2^{1/2} - 1)},\]

\[= 2 - 2^3,\]

for sufficiently large \(t\).

As can be be seen in Figure 3.2, initially the number of cells in the \(G_1\) compartment drops as there are cells leaving but none entering. The number of cells in the \(S\) compartment increases from zero, followed by the \(G_2\) and \(M\) compartments respectively. Once the number of cells in the \(M\) compartment is half that of the \(G_1\) compartment then the number of cells in \(G_1\) also starts to increase. Intuitively this seems to be the correct response but is easily verified by considering the stationary point of Equation (3.1a). To compare this result to that of Steel’s formulae it is necessary to obtain an estimate of the average time cells spend in a compartment. Equations (2.35-2.38) state that if there is no cell death \((\mu_i = 0 \forall i \in \{G_1, S, G_2, M\})\) then the average time a cell spends in a compartment is the reciprocal of the rate they leave that compartment. Since \(k_i = 1 \forall i \in \{1, 2, 3, 4\}\) the corresponding average time a cell spends in each of the compartments is also all one.
3.1 Structured Ordinary Differential Equation Model

Figure 3.2: Growth Curves for the Number of Cells in Different Phases as a Function of Time when $k_i = 1$, $i \in \{1, 2, 3, 4\}$ and $N_0 = 10^6$.

Figure 3.3: Fraction of the Total Population of Cells in Different Phases as a Function of Time when $k_i = 1$, $i \in \{1, 2, 3, 4\}$ and $N_0 = 10^6$. 
From Equations (2.13), (2.17) and (2.19) Steel’s formulae predicts the fractions to be

\[ P_{G2M} = 2^{\frac{1}{i}} - 1, \]
\[ P_S = 2^{\frac{1}{i}} - 2^{\frac{2}{i}}, \]
\[ P_{G1} = 1 - P_S - P_{G2M}, \]
\[ = 1 - \left(2^{\frac{2}{i}} - 2^{\frac{1}{i}}\right) - \left(2^{\frac{3}{i}} - 1\right), \]
\[ = 2 - 2^{\frac{4}{i}}, \] (3.14)

which agrees exactly with the values given by the ODE model prediction given by Equation (3.13). It should be noted that the predicted values from the ODE model only agree exactly with Steel’s formulae for very specific cases of \( k_i \). To find the maximum difference between the predicted values from the ODE models and Steel’s formula the difference was maximised using Matlab’s \([44]\) minimising function \( \text{fmincon} \). To maximise the function using \( \text{fmincon} \), the function \( g(k_i) = \text{difference} \) was minimised. The constraints placed on the parameter space were \( k_i \in [0.05, 2] \forall i \in \{1, 2, 3, 4\} \), which from Equations (2.35-2.38) correspond to average phase lengths of between 30 minutes and 20 hours. A range of starting values for the parameters was used ensuring the maximum obtained was the global maximum within the parameter space. The maximum difference in the predicted value for the percentage of cells in the \( G_1 \) phase between the ODE model and Steel’s formulae was found to be 3.7 %. The difference between the two methods being due in part to only the dominating \( R \) value of the characteristic polynomial being considered in the ODE model and the use of Equations (2.35-2.38) to obtain an estimate of the average time cells spend in a compartment.

Thus, the ODE model described may be adequate if the information required only concerns the number of cells in each phase. However, to adequately model heterogeneity of the the cells’ age, mass or DNA distribution within each phase a system of partial differential equations is needed.
3.2 A Simplified Age Structured Partial Differential Equation Model

In this section a new simplified age structured population balance model is presented. The purpose of this new model is twofold. Firstly it is to demonstrate that a simple model is sufficient to fit experimental data. Secondly by using a simpler model than those detailed in [22], [23] and [57] it is easier to gain insight into the model’s behaviour, particularly the transition of cells between the $G_1$ and $S$ phases, this is considered in detail in Section 3.3.

The new age structured model is based upon the CelCyMUS model discussed in Section 2.2.1. The CelCyMUS model may be simplified considerably by making several assumptions. These assumptions are

- **No flow**
  The system now being considered is closed such that no cells or nutrients enter or leave the system for $t > 0$, hence the flow rate $Q(t)$ is set to zero.

- **Constant glutamine uptake**
  If $\frac{dC_{GLUT}(t)}{dt} = \frac{dC_{GLUT}(t)}{\tau} = \frac{R_{GLUT}}{2}$ a direct comparison can now be made with [22] and [23] who use $\frac{dC_{GLUT}(t)}{dt} = R_{GLUT}$ along characteristic curves.

- **Reduction in number of transition rules**
  All of the end of phase transition rules, for example Equation (2.57) are removed and now included in the relevant boundary conditions.

- **Reduction in the number of phase/compartments**
  Phases $S$, $G_2$ and $M$ are all of fixed length and during these phases the cells do not absorb glutamine or leave via death so these three phases are combined into one compartment called ‘MAIN’. By combining the $S$, $G_2$ and $M$ phases the model is simplified from five partial differential equations to three. However, information about the distribution of the cells in these different phases is lost and as such must be inferred by the relative time length of these phases. It is also now more difficult to perturb the system by an amount dependent on cell phase.

In this model the cell cycle is divided into three, age structured compartments, $G_{1a}$, $G_{1b}$ and MAIN. The MAIN compartment contains cells in the $S$, $G_2$ and $M$ phases of the
cell cycle, division occurs at the end of this compartment. As this is an age-structured model the mechanisms and location of DNA replication are not important, however this is assumed to occur at the start of the \textit{MAIN} compartment. The $G_{1a}$ compartment contains cells which have just undergone division, i.e. new cells. Cells that are in $G_{1a}$ are not able to progress further round the cell cycle until a fixed time period has elapsed, this represents the minimum age a cell can start replicating its DNA. This is biologically realistic as new cells are normally unable to immediately start replicating their DNA. Once cells have progressed to the $G_{1b}$ compartment they undergo transition to the \textit{MAIN} compartment via some transition function $R_{2G_{1b}}(v)$ which is often a function of how long the cell has spent in the $G_{1b}$ compartment. It may also be a function of other factors which effect a cell’s progression round the cell cycle such as nutrient levels, the presence of certain drugs and temperature. The \textit{MAIN} compartment is of fixed duration and can be thought of as merely a time delay from cells leaving the $G_{1b}$ compartment until cell division occurs and the new cells enter into the $G_{1a}$ compartment. All compartments within this model are of a limited duration, the \textit{MAIN} and $G_{1a}$ compartments are of a fixed duration and the duration of $G_{1b}$ varies from zero to some maximum value, $T_{G_{1b}}$. Any cells remaining in the $G_{1b}$ compartment at the end of this period have not successfully undergone transition and are assumed to progress to a death phase, not modelled here, where the cells are no longer viable. In some cell lines cells may enter a quiescent phase where they remain viable but leave the cycle for a period of time, this may apply to cells who are still in the $G_{1b}$ compartment after a given time. These cells may be able to rejoin the cycle at a later time, this scenario is not included in this model.
Equations for the Simplified Model

The equations governing the cell populations in each of the compartments are now given by

**G\textsubscript{1a} Compartment Equation**

$$\frac{\partial}{\partial \tau} n_{G_{1a}}(t, \tau_{G_{1a}}) + \frac{\partial}{\partial t} n_{G_{1a}}(t, \tau_{G_{1a}}) = 0. \quad (3.15)$$

**G\textsubscript{1a} Compartment Initial Conditions**

$$n_{G_{1a}}(t, 0) = \begin{cases} 2n_{\text{MAIN}}(t, T_{\text{MAIN}}) & \text{for } C_{\text{GLUT}}(t) > 0, \\ 0 & \text{for } C_{\text{GLUT}}(t) = 0. \end{cases} \quad (3.16)$$
\( G_{1b} \) Compartment Equation

\[
\frac{\partial}{\partial t} n_{G_{1b}}(t, \tau_{G_{1b}}) + \frac{\partial}{\partial \tau} n_{G_{1b}}(t, \tau_{G_{1b}}) + R_{2G_{1b}}(n_{G_{1b}}(t, \tau_{G_{1b}}), \tau_{G_{1b}}) = 0, \tag{3.17}
\]

where

\[
R_{2G_{1b}}(n_{G_{1b}}(t, \tau_{G_{1b}}), \tau_{G_{1b}}) = \begin{cases} 
\frac{R_{\text{GLUT}} n_{G_{1b}}(t, \tau_{G_{1b}})}{S_{\text{MAX}} - C_{\text{GLUT}}(t, \tau_{G_{1b}})} & \text{for } C_{\text{GLUT}}(t) > 0, \\
0 & \text{for } C_{\text{GLUT}}(t) = 0. 
\end{cases} \tag{3.18}
\]

It should be noted that the transition function \( R_{2G_{1b}}(n_{G_{1b}}(t, \tau_{G_{1b}}), \tau_{G_{1b}}) \) given by Equation (3.18) is the same as that used in [22] and [23]. The factor of two being omitted because of the assumption \( \frac{\partial C_{\text{GLUT}}(t, \tau)}{\partial t} = \frac{\partial C_{\text{GLUT}}(t, \tau)}{\partial \tau} = \frac{R_{\text{GLUT}}}{2} \).

\( G_{1b} \) Compartment Initial Conditions

\[
n_{G_{1b}}(t, 0) = n_{G_{1a}}(t, T_{G_{1a}}) + n_{G'}(t, T_{G'}). \tag{3.19}
\]

\( \text{MAIN} \) Compartment Equation

\[
\frac{\partial}{\partial t} n_{\text{MAIN}}(t, \tau_{\text{MAIN}}) + \frac{\partial}{\partial \tau} n_{\text{MAIN}}(t, \tau_{\text{MAIN}}) = 0. \tag{3.20}
\]

\( \text{MAIN} \) Phase Initial Conditions

\[
n_{\text{MAIN}}(t, 0) = \begin{cases} 
\int_{0}^{T_{G_{1b}}} \frac{R_{\text{GLUT}} n_{G_{1b}}(t, \tau_{G_{1b}})}{S_{\text{MAX}} - C_{\text{GLUT}}(t, \tau_{G_{1b}})} d\tau_{G_{1b}} & \text{for } C_{\text{GLUT}}(t) > 0, \\
0 & \text{for } C_{\text{GLUT}}(t) = 0. 
\end{cases} \tag{3.21}
\]

Glutamine Equations

\[
\frac{dC_{\text{GLUT}}(t)}{dt} = -R_{\text{GLUT}} \left( \int_{0}^{T_{G_{1a}}} n_{G_{1a}}(t, \tau_{G_{1a}}) d\tau_{G_{1a}} + \int_{0}^{T_{G_{1b}}} n_{G_{1b}}(t, \tau_{G_{1b}}) d\tau_{G_{1b}} \right), \tag{3.22}
\]

where

\[
\frac{dC_{\text{GLUT}}(t, \tau_{G_{1b}})}{dt} = \begin{cases} 
R_{\text{GLUT}} & \text{for } C_{\text{GLUT}}(t) > 0, \\
0 & \text{for } C_{\text{GLUT}}(t) = 0. 
\end{cases} \tag{3.23}
\]

It should be noted that the derivatives in Equation (3.23) are full derivatives not partial derivatives for the reasons previously discussed in Section 2.2.1.
Non-Dimensionalisation of the Equations

In order to remove any potential numerical problems caused by very large and very small scales the system may be non-dimensionalised. By non-dimensionalising the system of equations it may be possible to reduce the number of dependent parameters making the system more tractable.

The first step in the non-dimensionalisation process is to introduce new, dimensionless variables \( \tilde{t}, \tilde{\tau}_{G'}^a, \tilde{\tau}_{G_1}, \tilde{\tau}_{MAIN}, \tilde{n}_{G'}^a(\tilde{t}, \tilde{\tau}_{G'}), \tilde{n}_{G_1a}(\tilde{t}, \tilde{\tau}_{G_1a}), \tilde{n}_{MAIN}(\tilde{t}, \tilde{\tau}_{MAIN}), \tilde{C}(\tilde{t}, \tilde{\tau}_{G_1b}) \) and \( \tilde{C}_{GLUT}(\tilde{t}) \). Since time and age progress at the same rate and have the same units (units of time) it seems sensible to scale all the ages and time by the same amount. It also seems sensible to scale the cell densities in the different compartments by the same rate. If this is not done this may lead to misleading phase distributions. \( C_{GLUT}(t, \tau_{G_1b}) \) and \( C_{GLUT}(t) \) are not scaled by the same amount as these refer to the intracellular and intercellular glutamine levels which will normally differ by several orders of magnitude. Hence we let

\[
\tilde{t} = \frac{t}{b},
\]

\[
\tilde{\tau}_{G'}^a = \frac{\tau_{G'}^a}{b},
\]

\[
\tilde{\tau}_{G_1} = \frac{\tau_{G_1}}{b},
\]

\[
\tilde{\tau}_{G_1b} = \frac{\tau_{G_1b}}{b},
\]

\[
\tilde{\tau}_{MAIN} = \frac{\tau_{MAIN}}{b},
\]

\[
\tilde{n}_{G'}^a(\tilde{t}, \tilde{\tau}_{G'}^a) = \frac{n_{G'}^a(t, \tau_{G'}^a)}{a},
\]

\[
\tilde{n}_{G_1a}(\tilde{t}, \tilde{\tau}_{G_1a}) = \frac{n_{G_1a}(t, \tau_{G_1a})}{a},
\]

\[
\tilde{n}_{G_1b}(\tilde{t}, \tilde{\tau}_{G_1b}) = \frac{n_{G_1b}(t, \tau_{G_1b})}{a},
\]
\[ \tilde{n}_{MAIN}(\tilde{t}, \tilde{\tau}_{MAIN}) = \frac{n_{MAIN}(t, \tau_{MAIN})}{a}, \]  
\[ \tilde{C}(\tilde{t}, \tilde{\tau}_{G_{1b}}) = \frac{C_{GGLUT}(t, \tau_{G_{1b}})}{c}, \]  
\[ \tilde{C}_{GLUT}(\tilde{t}) = \frac{C_{GLUT}(t)}{g}. \]  

Substituting the non-dimensional variables into Equations (3.15-3.23) gives

**G\textsubscript{1a} Compartment Equations**

\[ \frac{a}{b} \frac{\partial}{\partial \tilde{t}} \tilde{n}_{G_{1a}}(\tilde{t}, \tilde{\tau}_{G_{1a}}) + \frac{a}{b} \frac{\partial}{\partial \tilde{\tau}_{G_{1a}}} \tilde{n}_{G_{1a}}(\tilde{t}, \tilde{\tau}_{G_{1a}}) = 0. \]  

**G\textsubscript{1a} Compartment Initial Conditions**

\[ a\tilde{n}_{G_{1a}}(\tilde{t}, 0) = \begin{cases} 2a\tilde{n}_{MAIN}(\tilde{t}, \tilde{T}_{MAIN}) & \text{for } g\tilde{C}_{GLUT}(\tilde{t}) > 0, \\ 0 & \text{for } g\tilde{C}_{GLUT}(\tilde{t}) = 0. \end{cases} \]  

**G\textsubscript{1b} Compartment Equations**

\[ \frac{a}{b} \frac{\partial}{\partial \tilde{t}} \tilde{n}_{G_{1b}}(\tilde{t}, \tilde{\tau}_{G_{1b}}) + \frac{a}{b} \frac{\partial}{\partial \tilde{\tau}_{G_{1b}}} \tilde{n}_{G_{1b}}(t, \tau_{G_{1b}}) + R_{2G_{1b}}(\tilde{n}_{G_{1b}}(\tilde{t}, \tilde{\tau}_{G_{1b}}), \tilde{\tau}_{G_{1b}}) = 0, \]  

where

\[ R_{2G_{1b}}(a\tilde{n}_{G_{1b}}(\tilde{t}, \tilde{\tau}_{G_{1b}}), b\tilde{\tau}_{G_{1b}}) = \begin{cases} \frac{a_{RGLUT}\tilde{n}_{G_{1b}}(\tilde{t}, \tilde{\tau}_{G_{1b}})}{S_{MAX} - \tilde{C}(t, \tau_{G_{1b}})} & \text{for } g\tilde{C}_{GLUT}(\tilde{t}) > 0, \\ 0 & \text{for } g\tilde{C}_{GLUT}(\tilde{t}) = 0. \end{cases} \]  

Let \( \tilde{R}_{2G_{1b}}(\tilde{n}_{G_{1b}}(\tilde{t}, \tilde{\tau}_{G_{1b}}), \tilde{\tau}_{G_{1b}}) \) be defined as

\[ \tilde{R}_{2G_{1b}}(\tilde{n}_{G_{1b}}(\tilde{t}, \tilde{\tau}_{G_{1b}}), \tilde{\tau}_{G_{1b}}) = R_{2G_{1b}}(a\tilde{n}_{G_{1b}}(\tilde{t}, \tilde{\tau}_{G_{1b}}), b\tilde{\tau}_{G_{1b}}). \]  

Therefore

\[ \tilde{R}_{2G_{1b}}(\tilde{n}_{G_{1b}}(\tilde{t}, \tilde{\tau}_{G_{1b}}), \tilde{\tau}_{G_{1b}}) = \begin{cases} \frac{a_{RGLUT}\tilde{n}_{G_{1b}}(\tilde{t}, \tilde{\tau}_{G_{1b}})}{S_{MAX} - \tilde{C}(t, \tau_{G_{1b}})} & \text{for } g\tilde{C}_{GLUT}(\tilde{t}) > 0, \\ 0 & \text{for } g\tilde{C}_{GLUT}(\tilde{t}) = 0. \end{cases} \]  

**G\textsubscript{1b} Compartment Initial Conditions**

\[ a\tilde{n}_{G_{1b}}(\tilde{t}, 0) = a\tilde{n}_{G_{1a}}(\tilde{t}, \tilde{T}_{G_{1a}}) + a\tilde{n}_{G_{1b}}(\tilde{t}, \tilde{T}_{G_{1b}}). \]
3.2 A Simplified Age Structured Partial Differential Equation Model

**MAIN Compartment Equations**

\[
\frac{a}{b} \frac{\partial}{\partial t} \tilde{n}_{\text{MAIN}}(\tilde{t}, \tilde{n}_{\text{MAIN}}) + \frac{a}{b} \frac{\partial}{\partial \tau} \tilde{n}_{\text{MAIN}}(\tilde{t}, \tilde{n}_{\text{MAIN}}) = 0. \tag{3.32}
\]

**MAIN Compartment Initial Conditions**

\[
a \tilde{n}_{\text{MAIN}}(\tilde{t}, 0) = \begin{cases} \tilde{r}_{G1b} \frac{abR_{\text{GLUT}} \tilde{n}_{G1b}(\tilde{t}, \tilde{n}_{G1b})}{S_{\text{MAX}} - c(t, \tilde{n}_{G1b})} d\tilde{n}_{G1b} & \text{for } g \tilde{C}_{\text{GLUT}}(\tilde{t}) > 0, \\ 0 & \text{for } g \tilde{C}_{\text{GLUT}}(\tilde{t}) = 0. \end{cases} \tag{3.33}
\]

Equations (3.25-3.33) may be simplified by choosing \( c = S_{\text{MAX}} \) and \( b = \frac{S_{\text{MAX}}}{R_{\text{GLUT}}} \). Note that \( a \) may be chosen arbitrarily, so to normalise the total cell count it is chosen to equal the starting number of cells, \( a = \int_0^T c(t) n(t, \tau) d\tau \). Equations (3.25-3.33) now become

**G_{1a} Compartment Equations**

\[
\frac{\partial}{\partial t} \tilde{n}_{G1a}(\tilde{t}, \tilde{n}_{G1a}) + \frac{\partial}{\partial \tau} \tilde{n}_{G1a}(\tilde{t}, \tilde{n}_{G1a}) = 0. \tag{3.34}
\]

**G_{1a} Compartment Initial Conditions**

\[
\tilde{n}_{G1a}(\tilde{t}, 0) = \begin{cases} 2 \tilde{n}_{\text{MAIN}}(\tilde{t}, \tilde{T}_{\text{MAIN}}) & \text{for } g \tilde{C}_{\text{GLUT}}(\tilde{t}) > 0, \\ 0 & \text{for } g \tilde{C}_{\text{GLUT}}(\tilde{t}) = 0. \end{cases} \tag{3.35}
\]

**G_{1b} Compartment Equations**

\[
\frac{\partial}{\partial t} \tilde{n}_{G1b}(\tilde{t}, \tilde{n}_{G1b}) + \frac{\partial}{\partial \tau} \tilde{n}_{G1b}(\tilde{t}, \tilde{n}_{G1b}) + \hat{R}_{2G1b}(\tilde{n}_{G1b}(\tilde{t}, \tilde{n}_{G1b}), \tilde{n}_{G1b}) = 0, \tag{3.36}
\]

where

\[
\hat{R}_{2G1b}(\tilde{n}_{G1b}(\tilde{t}, \tilde{n}_{G1b}), \tilde{n}_{G1b}) = \begin{cases} \frac{\tilde{n}_{G1b}(\tilde{t}, \tilde{n}_{G1b})}{1 - \tilde{n}_{G1b}(\tilde{t}, \tilde{n}_{G1b})} & \text{for } g \tilde{C}_{\text{GLUT}}(\tilde{t}) > 0, \\ 0 & \text{for } g \tilde{C}_{\text{GLUT}}(\tilde{t}) = 0. \end{cases} \tag{3.37}
\]

**G_{1b} Compartment Initial Conditions**

\[
\tilde{n}_{G1b}(\tilde{t}, 0) = \tilde{n}_{G1a}(\tilde{t}, \tilde{T}_{G1a}) + \tilde{n}_{G1b}(\tilde{t}, \tilde{T}_{G1b}). \tag{3.38}
\]

**MAIN Compartment Equations**

\[
\frac{\partial}{\partial t} \tilde{n}_{\text{MAIN}}(\tilde{t}, \tilde{n}_{\text{MAIN}}) + \frac{\partial}{\partial \tau} \tilde{n}_{\text{MAIN}}(\tilde{t}, \tilde{n}_{\text{MAIN}}) = 0. \tag{3.39}
\]
\( \text{MAIN Compartment Initial Conditions} \)

\[
\tilde{n}_{\text{MAIN}}(\tilde{t}, 0) = \begin{cases} 
\int_{0}^{\tilde{T}_{G_{1b}}} \frac{\tilde{\tilde{n}}_{G_{1b}}(\tilde{t}, \tilde{\tau}_{G_{1b}})}{1 - \tilde{C}(\tilde{t}, \tilde{\tau}_{G_{1b}})} d\tilde{\tau}_{G_{1b}} & \text{for } g\tilde{C}_{\text{GLUT}}(\tilde{t}) > 0, \\
0 & \text{for } g\tilde{C}_{\text{GLUT}}(\tilde{t}) = 0.
\end{cases}
\] (3.40)

In order to choose \( g \) the equation for the intercellular glutamine given by Equation (3.22) is changed into a non dimensional form. Using the substitutions given in Equations (3.24a, 3.24c, 3.24d, 3.24g, 3.24h and 3.24k) into Equation (3.22) gives

\[
\frac{g}{b} \frac{d\tilde{C}_{\text{GLUT}}(\tilde{t})}{dt} = -aR_{\text{GLUT}} \left( \int_{0}^{\tilde{T}_{G_{1a}}} \tilde{\tilde{n}}_{G_{1a}}(\tilde{t}, \tilde{\tau}_{G_{1a}}) d\tilde{\tau}_{G_{1a}} + \int_{0}^{\tilde{T}_{G_{1b}}} \tilde{\tilde{n}}_{G_{1b}}(\tilde{t}, \tilde{\tau}_{G_{1b}}) d\tilde{\tau}_{G_{1b}} \right), \tag{3.41}
\]

i.e.

\[
\frac{d\tilde{C}_{\text{GLUT}}(\tilde{t})}{dt} = -\frac{ab}{g} R_{\text{GLUT}} \left( \int_{0}^{\tilde{T}_{G_{1a}}} \tilde{\tilde{n}}_{G_{1a}}(\tilde{t}, \tilde{\tau}_{G_{1a}}) d\tilde{\tau}_{G_{1a}} + \int_{0}^{\tilde{T}_{G_{1b}}} \tilde{\tilde{n}}_{G_{1b}}(\tilde{t}, \tilde{\tau}_{G_{1b}}) d\tilde{\tau}_{G_{1b}} \right). \tag{3.42}
\]

It should be noted the constant \( R_{\text{GLUT}} \) defined in Equation (3.23) is not rescaled. A natural choice for \( g \) is therefore \( g = abR_{\text{GLUT}} = S_{\text{MAX}} \int_{0}^{\tilde{T}_{G_{1a}}} n_{\text{GLUT}}(0, \tau) d\tau \).

The non-dimensionalisation that has been described in this section forms the basis for any non-dimensionalisation which will be used. It is clear from the non dimensionalisation that the form of the transition function plays an important role in the choice of the appropriate scaling constants. As such a specific non-dimensionalisation will be briefly outlined for the new transition function detailed in Section 3.3.

### 3.3 Transition Functions

In the literature there are a number of different functions describing the transition of cells between the \( G_{1} \) and \( S \) phases of the cell cycle ([6], [22], [23] and [57]). In this section two different approaches for deriving the transition function are detailed. A constant transition function of the form used in [6] is then briefly discussed. The quadratic and sigmoidal functions used in ([22], [23] and [57]) are then discussed in detail, with respective flaws highlighted. A new, sigmoidal transition function is then presented together with the appropriate non-dimensionalisation of the system.
3.3.1 Derivation of Transition Functions

The probability of a cell leaving the $G_{1b}$ phase and entering the $S$ phase via the transition rule is given by some probability distribution function $f(x)$ where $x$ is the variable that determines how likely cells are to undergo transition, for example, age, mass or concentration of some nutrient. Figure 3.5 gives a graphical representation of a typical biologically reasonable probability distribution function with phase age $\tau_{G_{1b}}$ acting as the variable controlling the transition probability. Figure 3.5 shows that the probability of transition is low for cells that have only recently joined the $G_{1b}$ phase, as they have not had the time to acquire the nutrients needed to successfully complete the cycle. The probability then peaks for a range of $\tau_{G_{1b}}$ and tails off for cells which have been in the $G_{1b}$ phase for a long period of time. Note that phase age is the length of time a cell spends in a particular phase. For the rest of this derivation the subscripts have been removed from the age variable for ease. If $\tau$ varies by a small amount, $\delta \tau$, then the probability of cells

\[
\frac{dP}{d\tau} = -f(\tau)\delta \tau
\]

whose age is in $[\tau, \tau + \delta \tau)$ transitioning can be approximated by $f(\tau)\delta \tau$. Assuming all cells are capable of transitioning given enough nutrients, the total area under the probability distribution curve is one. Therefore the probability that a cell of age $\tau$ has not yet transitioned is given by $1 - \int_{0}^{\tau} f(\tau')d\tau'$. So the fraction of cells, who have not gone through transition, who go through transition when their age changes from $\tau$ to $\tau + \delta \tau$ is

\[
P(\tau) = 1 - \int_{0}^{\tau} f(\tau')d\tau'
\]

Figure 3.5: Probability Distribution of Transition, $f(\tau)$ Showing the Probability that a Cell of Age $\tau$ has not yet Transitioned (Shaded Region) and the Probability a Cell of Age $\tau$ will Transition in the Time Interval $\tau$ to $\tau + \delta \tau$ (Dark Region).
given by
\[ f(\tau)\delta\tau \] \(\frac{1}{1 - \int_0^\infty f(\tau')d\tau'}\). \hspace{1cm} (3.43)

Another way of considering the number of cells going through transition is via a transition rate \(h(\tau)\). The fraction of cells who leave in the time period \([t, t + \delta t]\) is given by rate cells leave \(\times\) time i.e \(h(\tau)\delta t\), then by definition this must be equal to Equation (3.43). Therefore,
\[ h(\tau) = \frac{f(\tau)\delta\tau}{1 - \int_0^\infty f(\tau')d\tau'} \] \hspace{1cm} (3.44)

which, in the limit \(\delta t \to 0\), becomes
\[ h(\tau) = \frac{f(\tau)\delta\tau}{1 - \int_0^\infty f(\tau')d\tau'} \] \hspace{1cm} (3.45)

Since a cell ages at the same rate as time passes \(\tau(t) = t - c\) where \(c\) is a constant therefore \(\frac{d\tau}{dt} = 1\) hence Equation (3.45) simplifies to
\[ h(\tau) = \frac{f(\tau)}{1 - \int_0^\infty f(\tau')d\tau'} \] \hspace{1cm} (3.46)

If the cumulative probability of cells transitioning, \(F(\tau)\), is considered instead of the probability distribution, \(f(\tau)\), then Equation (3.46) may be expressed as
\[ h(\tau) = \frac{F'(\tau)}{1 - F(\tau)} \] \hspace{1cm} (3.47)

where the dash notation denotes the derivative with respect to \(\tau\). It is this form of the transition rate which is most frequently used and will be used herein.

An alternative approach for deriving the transition rate is detailed in [32]. The material leaving in the time period \([t, t + \delta t]\) is equal to the flow rate \(Q(t)\) multiplied by the time interval \(\delta t\) multiplied by the age distribution of the exit stream \(E(t)\). Clearly this must be equal to the volume \(V\) multiplied by the age distribution of the material which has not left prior to time \(t\), \(I(\tau) = 1 - \int_0^\tau f(\tau')d\tau'\) multiplied by the fraction which will leave over the time period \(\delta t\) i.e. \(\Lambda(\tau)\delta t\). Thus,

\[ \text{Flow Rate} \times \text{time interval} \times \text{age distribution of exit stream} = \] \hspace{1cm} (3.48)

\[ \text{Volume} \times \text{age distribution of population} \times \text{fraction that will leave over } \delta t, \]
or, using the same notation as [32],

\[ Q(t) \times E(\tau) \times \delta t = V \times I(\tau) \times \Lambda(\tau) \delta t. \] (3.49)

In this notation \( \Lambda(\tau) \) can be thought of as the transition rate for material of age \( \tau \). Also, if the flow is constant and normalised such that \( Q/V = 1 \) Equation (3.49) simplifies to

\[ \Lambda(\tau) = \frac{E(\tau)}{I(\tau)} \] (3.50)

where \( E(\tau) \) corresponds to the probability distribution \( f(\tau) \). Since \( I(\tau) = 1 - \int_0^T f(\tau')d\tau' \) Equations (3.47) and (3.50) are equivalent.

3.3.2 Constant Transition Function

In [6] a constant transition function, \( h = c \), is used at the \( G_1 - S \) phase checkpoint. Such a transition function is therefore independent of the time spent in the \( G_{1b} \) phase and is therefore not biologically realistic as it implies all cells in the \( G_{1b} \) phase have an equal probability of progressing to the \( S \) phase regardless of how long they have spent acquiring nutrients and preparing for DNA synthesis. However, the use of such a transition function may be justified and the effects of using a constant transition function are discussed further in Section 3.5.1 To obtain the cumulative probability distribution \( F(\tau) \) for a constant transition function it is necessary to substitute \( h = c \) into Equation (3.47) which gives

\[ c = \frac{F'(\tau)}{1 - F(\tau)}. \] (3.51)

Assuming that the cumulative probability is zero at a phase age of zero this differential equation for \( F(\tau) \) may readily be solved to give

\[ F(\tau) = 1 - e^{\tau}. \] (3.52)

3.3.3 Quadratic Transition Function

In [22] the rate of transition is given as the derivative of a function defined as the fraction of cells whose phase age was 0 at time \( t - \tau_{G_{1b}} \) that still remain in \( G_{1b} \) at time \( t \) (note these cells now have a cell age of \( \tau_{G_{1b}} \)). This is given as a function of intracellular glutamine \( C_{c1G_{1b}}(t, \tau_{G_{1b}}) \). The expression for this function is defined as

\[ \frac{n_{G_{1b}}(t, \tau_{G_{1b}})}{n_{G_{1b}}(t - \tau_{G_{1b}}, 0)} = \left( \frac{C_{c1G_{1b}}(t, \tau_{G_{1b}}) - S_{Max}}{S_{Max}^2} \right)^2, \] (3.53)
where $S_{Max}$ is the maximum glutamine content a cell may have before being forced to go through transition. From Equation (3.53) it follows that the cumulative probability of transition, $F(t, \tau_{G1b})$, is given by

$$F(t, \tau_{G1b}) = 1 - \frac{(C_{c1G1b}(t, \tau_{G1b}) - S_{Max})^2}{S_{Max}^2}. \quad (3.54)$$

A plot of this cumulative probability function for an arbitrary $t$ is shown in Figure 3.6. From Equation (3.54) it follows

![Figure 3.6: Sketch of a Quadratic Cumulative Probability Distribution Function for an Arbitrary Time for the Transition Function Presented in [22].](image)

$$\frac{\partial F}{\partial \tau_{G1b}} = -\frac{2(C_{c1G1b}(t, \tau_{G1b}) - S_{Max})}{S_{Max}^2} \frac{\partial C_{c1G1b}(t, \tau_{G1b})}{\partial \tau_{G1b}}. \quad (3.55)$$

Substituting Equations (3.54) and (3.55) into Equation (3.47) yields

$$h(t, \tau_{G1b}) = \frac{2(C_{c1G1b}(t, \tau_{G1b}) - S_{Max})}{(C_{c1G1b}(t, \tau_{G1b}) - S_{Max})^2} \frac{\partial C_{c1G1b}(t, \tau_{G1b})}{\partial \tau_{G1b}},$$

$$= \frac{2}{S_{Max} - C_{c1G1b}(t, \tau_{G1b})} \frac{\partial C_{c1G1b}(t, \tau_{G1b})}{\partial \tau_{G1b}}. \quad (3.56)$$
This differs by a minus sign from the expression in [22] however, it can be seen that this version is correct since \( h(t, \tau_{Gib}) \) is now \( \geq 0 \) for all \( C_{clGib}(t, \tau_{Gib}) \). It is assumed that the intracellular glutamine never decreases, hence \( \frac{\partial C_{clGib}(t, \tau_{Gib})}{\partial \tau_{Gib}} \) is always \( \geq 0 \). It is worth noting that if the glutamine in the extra cellular medium is sufficiently abundant the uptake rate will be constant, and as such the intracellular glutamine and transition rate will now only be functions of cell age.

This transition rule has the major flaw that when \( C_{clGib}(t, \tau_{Gib}) = S_{Max} \), which is a biologically realistic possibility, it is undefined, this problem was mentioned in [57], who proposed an alternative transition function.

To obtain the transition rule given in [22] it is possible to work backwards, allowing the corresponding cumulative probability distribution to be found. By substituting \( h(t, \tau_{Gib}) = \frac{C_{clGib}^2(t, \tau_{Gib}) - S_{Max}}{c_{clGib}(t, \tau_{Gib}) - S_{Max}} \) into Equation (3.47) then solving the corresponding differential equation for \( F(t, \tau_{Gib}) \) gives a cumulative probability distribution of

\[
F(t, \tau_{Gib}) = 1 - \left( \frac{S_{Max}}{S_{Max} - C_{clGib}(t, \tau_{Gib})} \right)^2.
\]  

Figure 3.7 shows a plot of this function. Since the cumulative probability distribution must always be positive and monotonically increasing this function is incorrect.

### 3.3.4 Sigmoidal Transition Function

The problems arising from the quadratic transition function tending to infinity as \( C_{clGib}(t, \tau_{Gib}) \to S_{Max} \) were addressed by [57] who proposed a sigmoidal shaped cumulative probability function for the transition of cells between the \( G_1 \) and \( S \) phases of the cycle.

In [57] the rate of transition is given as the derivative of a function defined as the fraction of cells whose phase age was 0 at time \( t - \tau_{Gib} \) that still remain in \( G_{ib} \) at time \( t \). The expression for this function is defined as

\[
\frac{n_{Gib}(t, \tau_{Gib})}{n_{Gib}(t - \tau_{Gib}, 0)} = 1 - \frac{1}{1 + e^{\alpha_{Gib}[S-d_{Gib}]}}.
\]  

where \( \alpha_{Gib} \) and \( d_{Gib} \) are parameters. It is not stated explicitly in [57] but it is implied that \( S \) used in [57] is the same as \( C_c \) used in [22], this is the intracellular glutamine and corresponds to \( C_c \) and \( C_{CGU} \) used in Section 2.2.1. As such, it is assumed \( S \) is
dependent on cell phase age $\tau_{i\ell b}$ and possibly time, although this is not in the formula given in [57]. If $S$ is meant to be constant this will make the cumulative fraction of cells transitioning constant and hence the transition rate will be zero. For clarity $C_{i\ell}(t, \tau_{i\ell b})$ will replace $S(t, \tau_{i\ell b})$ and the glucose concentration in the medium will be denoted by $C_{\text{Med}}(t)$.

In [57] the right hand side of Equation (3.58) is used as the cumulative probability distribution function $F$. However the left hand side of this equation relates to the fraction of cells of age $\tau_{i\ell b}$ remaining in the $G_{i\ell b}$ phase, $1 - F$. This can easily be shown by setting $\tau_{i\ell b}$ and $C_{i\ell}(t, \tau_{i\ell b})$ to zero in Equation (3.58) giving

$$\frac{n_{G_{i\ell b}}(t, 0)}{n_{G_{i\ell b}}(t, 0)} = 1 - \frac{1}{1 + e^{-\alpha_{i\ell b}d_{G_{i\ell b}}}}. \quad (3.59)$$

clearly the left hand side is one, for all values of $\alpha_{G_{i\ell b}}$ and $d_{G_{i\ell b}}$ used in [57] these are both strictly non zero and positive hence for Equation (3.59), LHS $\neq$ RHS. It is therefore
3.3 Transition Functions

assumed Equation (3.58) should read

\[ \frac{n_{G_{ib}}(t, \tau_{G_{ib}})}{n_{G_{ib}}(t - \tau_{G_{ib}}, 0)} = \frac{1 + e^{-\alpha_{G_{ib}}d_{G_{ib}}}}{1 + e^{\alpha_{G_{ib}}(C_c(t, \tau_{G_{ib}}) - d_{G_{ib}})}} \]  

(3.60)
giving the cumulative probability of transition as

\[ F(t, \tau_{G_{ib}}) = 1 - \frac{1 + e^{-\alpha_{G_{ib}}d_{G_{ib}}}}{1 + e^{\alpha_{G_{ib}}(C_c(t, \tau_{G_{ib}}) - d_{G_{ib}})}}. \]  

(3.61)
The transition function, \( h(t, \tau_{G_{ib}}) \), is readily obtained by substituting (3.61) and its derivative into Equation (3.47) to give

\[ h(t, \tau_{G_{ib}}) = \frac{\alpha_{G_{ib}} e^{\alpha_{G_{ib}}(C_c(t, \tau_{G_{ib}}) - d_{G_{ib}})}}{1 + e^{\alpha_{G_{ib}}(C_c(t, \tau_{G_{ib}}) - d_{G_{ib}})}} \frac{\partial C_c(t, \tau_{G_{ib}})}{\partial \tau_{G_{ib}}} \]  

(3.62)

Upon careful scrutiny of the FORTRAN code for the model presented in [57] it can be seen that this is not the function actually used. Instead,

\[ T_r = h(t, \tau_{G_{ib}}) = 1 - \frac{1}{1 + e^{\alpha_{G_{ib}}(C_c(t, \tau_{G_{ib}}) - d_{G_{ib}})}} \]  

(3.63)
this is the function used as \( F(t, \tau_{G_{ib}}) \) in the main body of the text. To check the validity of the transition function \( h(t, \tau_{G_{ib}}) \) used in the FORTRAN code it is necessary to rearrange the formulae

\[ T_r = 1 - \frac{1}{1 + e^{\alpha_{G_{ib}}(C_c(t, \tau_{G_{ib}}) - d_{G_{ib}})}} \]  

(3.64)
which differs from Equation (3.62) only by a factor of \( \alpha \frac{\partial C_c(t, \tau_{G_{ib}})}{\partial \tau_{G_{ib}}} \). Because it only differs by a factor this may still be a valid transition rule. To check the validity of this rule it is necessary to specify \( C_c(t, \tau_{G_{ib}}) \). It is reasonable to assume that \( C_c(t, \tau_{G_{ib}}) \) takes the same form as described in Equation (2.42), i.e. \( C_c(t, \tau_{G_{ib}}) = R\tau_{G_{ib}} \) where \( R \) is a constant. Making this assumption simplifies Equation (3.63) to

\[ h(\tau_{G_{ib}}) = 1 - \frac{1}{1 + e^{\alpha_{G_{ib}}(R\tau_{G_{ib}} - d_{G_{ib}})}}. \]  

(3.65)
Figure 3.8: A Plot of $F(\tau_{G_{1b}})$ as Defined in Equation (3.61) with $C_c(t, \tau_{G_{1b}}) = R\tau_{G_{1b}}$

Where $R$ is a Constant for $R = 0.1$, $d_{G_{1b}} = 1$ and $\alpha_{G_{1b}} = 5$. 

Figure 3.9: A Plot of $F(\tau_{G_{1b}})$ as Defined in Equation (3.67) for $R = 0.1$, $d_{G_{1b}} = 1$ and $\alpha_{G_{1b}} = 5$. 
Substituting for \( h(\tau_{G_{ib}}) \) from Equation (3.65) into Equation (3.47) and rearranging gives the differential equation

\[
\frac{dF(\tau_{G_{ib}})}{d\tau_{G_{ib}}} = (1 - F(\tau_{G_{ib}})) \left( 1 - \frac{1}{1 + e^{\alpha_{G_{ib}}[R\tau_{G_{ib}} - d_{G_{ib}}]}} \right).
\]

(3.66)

Solving Equation (3.66) together with the initial condition \( F(0) = 0 \) yields

\[
F(\tau_{G_{ib}}) = 1 - \left\{ \frac{1 + e^{-\alpha_{G_{ib}}d_{G_{ib}}}}{1 + e^{\alpha_{G_{ib}}[R\tau_{G_{ib}} - d_{G_{ib}}]}} \right\} \frac{1}{\alpha_{G_{ib}}}. \]

(3.67)

The function \( F(\tau_{G_{ib}}) \) now goes from zero to one and is therefore a valid cumulative distribution. Thus, despite the inconsistency between the FORTRAN code and the main body of text [57] uses a valid transition function. Plots of \( F(\tau_{G_{ib}}) \) as defined in Equation (3.61) with \( C_{c}(t, \tau_{G_{ib}}) = R\tau_{G_{ib}} \) where \( R \) is a constant and \( F(\tau_{G_{ib}}) \) as defined in Equation (3.67) are shown in Figures 3.8 and Figures 3.9 for the same parameter values.

### 3.3.5 New Sigmoidal Transition Function

The sigmoidal form of the transition rule seems biologically reasonable as it is sensible to assume the probability of cells progressing to the \( S \) phase immediately after entering the \( G_{ib} \) portion of the \( G_{1} \) phase is low due to the limited amount of nutrients they have taken up. Once the amount of nutrients taken up reaches some critical value then the probability of transition is likely to increase considerably, however there will always be a few cells which do not progress to the \( S \) phase regardless of nutrient uptake. Furthermore, a sigmoidal cumulative probability function is in keeping with the phase transition seen in cell populations which have been modelled using the kinetics and chemical processes within the cell [49] and [53]. A new sigmoidal transition rule is therefore proposed which, unlike the one considered in [57], may be non-dimensionalised so there is only one independent parameter which needs to be fitted, making the problem more tractable.

Consider a cumulative distribution function given by

\[
F(t, \tau_{G_{ib}}) = 1 - \frac{1 + e^{-\theta}}{1 + e^{\left( \frac{C_{c}(t, \tau_{G_{ib}})}{S} - 1 \right)/\tau}},
\]

(3.68)

where \( \theta \) and \( S \) are parameters which need to be fitted. Substituting Equation (3.68) into
Equation (3.47) gives
\[ h(t, \tau_{G_{ib}}) = \frac{\theta}{S} \frac{\partial C_c(t, \tau_{G_{ib}})}{\partial \tau_{G_{ib}}} \frac{e^{\left(\frac{C_c(t, \tau_{G_{ib}})}{S} - \frac{1}{2}\right)}}{1 + e^{\left(\frac{C_c(t, \tau_{G_{ib}})}{S} - \frac{1}{2}\right)}}. \]  
(3.69)

As with the other transition rules discussed previously it is reasonable to assume that the rate of change of intracellular glutamine is constant, provided there is a high amount of glutamine available. The case of limited glutamine availability is considered in Appendix A. By making the assumption that the rate of change of intracellular glutamine is constant and \( C_c(t, \tau_{G_{ib}}) = 0 \) at \( \tau_{G_{ib}} = 0 \), then \( \frac{\partial C_c(t, \tau_{G_{ib}})}{\partial \tau_{G_{ib}}} = R \) and \( C_c(t, \tau_{G_{ib}}) = R \tau_{G_{ib}} \). Equation (3.69) is now simplified to
\[ h(\tau_{G_{ib}}) = \frac{R \theta}{S} \frac{e^{\left(\frac{R \tau_{G_{ib}}}{S} - \frac{1}{2}\right)}}{1 + e^{\left(\frac{R \tau_{G_{ib}}}{S} - \frac{1}{2}\right)}}. \]  
(3.70)

In this transition function \( \theta \) is related to the maximum value of the cumulative distribution function and \( S \) is related to the steepness of the sigmoidal function.

Non-dimensionalisation Using the New Transition Function

The choice of the new variables obtained in the non-dimensionalisation process, previously discussed, depend largely on the transition function. As such, it is sufficient to only consider the non-dimensionalisation of the equations for the \( G_{ib} \) part of the \( G_1 \) phase to determine the new variables. For the \( G_{ib} \) compartment the equation governing the cell density is given by Equation (3.17), which using the transition function given in Equation (3.70), becomes
\[ \frac{\partial}{\partial t} n_{G_{ib}}(t, \tau_{G_{ib}}) + \frac{\partial}{\partial \tau} n_{G_{ib}}(t, \tau_{G_{ib}}) = -\frac{R \theta}{S} \frac{e^{\left(\frac{R \tau_{G_{ib}}}{S} - \frac{1}{2}\right)}}{1 + e^{\left(\frac{R \tau_{G_{ib}}}{S} - \frac{1}{2}\right)}} n_{G_{ib}}. \]  
(3.71)

Upon making the substitutions given by Equations (3.24b, 3.24d and 3.24h) this becomes
\[ \frac{a \hat{c}}{b \hat{c}} \frac{\partial}{\partial t} \tilde{n}_{G_{ib}}(\hat{t}, \tilde{\tau}_{G_{ib}}) + \frac{a \hat{c}}{b \hat{c}} \frac{\partial}{\partial \tilde{\tau}} \tilde{n}_{G_{ib}}(\hat{t}, \tilde{\tau}_{G_{ib}}) = -\frac{R \theta}{S} \frac{e^{\left(\frac{R \tilde{\tau}_{G_{ib}}}{S} - \frac{1}{2}\right)}}{1 + e^{\left(\frac{R \tilde{\tau}_{G_{ib}}}{S} - \frac{1}{2}\right)}} a \tilde{n}_{G_{ib}}, \]  
(3.72)
i.e.
\[
\frac{\partial}{\partial t}\tilde{n}_{G_{ib}}(\tilde{t}, \tilde{\tau}_{G_{ib}}) + \frac{\partial}{\partial \tau}\tilde{n}_{G_{ib}}(\tilde{t}, \tilde{\tau}_{G_{ib}}) = -\frac{Rb\theta}{S} e^{\theta\left(\frac{\tilde{n}_{G_{ib}}-\frac{\tilde{\tau}_{G_{ib}}}{2}}{\frac{\tilde{n}_{G_{ib}}-\frac{\tilde{\tau}_{G_{ib}}}{2}}{2}}\right)}\tilde{n}_{G_{ib}}. \tag{3.73}
\]
By setting \( b = \frac{s}{R\theta} \) this simplifies to
\[
\frac{\partial}{\partial t}\tilde{n}_{G_{ib}}(\tilde{t}, \tilde{\tau}_{G_{ib}}) + \frac{\partial}{\partial \tau}\tilde{n}_{G_{ib}}(\tilde{t}, \tilde{\tau}_{G_{ib}}) = -\frac{e^{\left(\frac{\tilde{n}_{G_{ib}}-\frac{\tilde{\tau}_{G_{ib}}}{2}}{\frac{\tilde{n}_{G_{ib}}-\frac{\tilde{\tau}_{G_{ib}}}{2}}{2}}\right)}}{1 + e^{\left(\frac{\tilde{n}_{G_{ib}}-\frac{\tilde{\tau}_{G_{ib}}}{2}}{\frac{\tilde{n}_{G_{ib}}-\frac{\tilde{\tau}_{G_{ib}}}{2}}{2}}\right)}}\tilde{n}_{G_{ib}}. \tag{3.74}
\]

3.4 Numerical Scheme

The system of differential equations governing the simplified system described in Section 3.2 may be solved analytically for specific initial conditions and short time intervals. However, in order to be able to study and manipulate the model for different transition functions for longer time intervals involving many cell cycles it is necessary to use numerical techniques.

Derivation of Numerical Scheme

In this section a finite difference scheme analogous to the Lax-Wendroff scheme is derived. The Lax-Wendroff scheme was chosen as it is a second order explicit method and as such yields high accuracy for relatively large time steps where there is a rapid change or discontinuity such as the initial flow of cells into the main cycle. The non dimensionalised form of the Equation (3.17) may be written as
\[
n_t + n_{\tau} = -hn. \tag{3.75}
\]
Note for ease the time and age dependence has been omitted together with the phase subscript. Subscripts now denote the partial derivatives. Also \( h \) is a assumed to be a function of \( \tau \) only. Rearranging and differentiating Equation (3.75) gives
\[
n_t = -n_{\tau} - hn, \tag{3.76a}
\]
\[
n_{tt} = -n_{\tau\tau} - h n_t, \tag{3.76b}
\]
\[
n_{tt\tau} = -n_{\tau\tau\tau} - h_{\tau\tau} n_{\tau} - h_{\tau} n. \tag{3.76c}
\]
The Taylor expansion of \( n \) is given by

\[
n(t + \delta t, \tau) = n + \delta t n_t + \frac{(\delta t)^2}{2} n_{tt} + O((\delta t)^3),
\]  

(3.77)

which, on substitution from Equations (3.76), becomes

\[
n(t + \delta t, \tau) = n + \delta t (-n_\tau - hn_t) + \frac{(\delta t)^2}{2} (-n_{t\tau} - hn_{tt}) + O((\delta t)^3),
\]  

(3.78)

i.e.

\[
n(t + \delta t, \tau) = n + \delta t (-n_\tau - hn_t) + \frac{(\delta t)^2}{2} (n_{t\tau} + 2hn_r + h^2 n + h_r n) + O((\delta t)^3).
\]  

(3.79)

### Choice of the Transition Function, \( h \)

The non-dimensionalised form of the new transition rule has been chosen for use in this numerical scheme,

\[
h = \frac{e^{(\tau - \frac{\eta}{2})}}{1 + e^{(\tau - \frac{\eta}{2})}}.
\]  

(3.80)

It is also worth noting that

\[
h_\tau = \frac{e^{(\tau - \frac{\eta}{2})}}{1 + e^{(\tau - \frac{\eta}{2})}} - \frac{\left\{ e^{(\tau - \frac{\eta}{2})} \right\}^2}{\left\{ 1 + e^{(\tau - \frac{\eta}{2})} \right\}^2} = h - h^2.
\]  

(3.81)

Substituting for \( h \) and its derivative into Equation (3.79) and simplifying gives

\[
n(t + \delta t, \tau) = n - \delta t (n_\tau + hn) + \frac{(\delta t)^2}{2} (n_{t\tau} + 2hn_r + h^2 n + h_r n) + O((\delta t)^3).
\]  

(3.82)

Collecting the derivatives of \( n \) together this becomes

\[
n(t + \delta t, \tau) = n \left( 1 - \delta t h + \frac{(\delta t)^2}{2} h \right) + n_\tau (-\delta t + (\delta t)^2 h) + n_{t\tau} \frac{(\delta t)^2}{2} + O((\delta t)^3).
\]  

(3.83)

Finally, standard formulae for the first and second derivatives of \( n \) with respect to \( \tau \) are used, namely

\[
\frac{\delta n}{\delta \tau}_{i,j} = \frac{n_{i,j+1} - n_{i,j-1}}{2\delta \tau},
\]  

(3.84)

\[
\frac{\delta^2 n}{\delta \tau^2}_{i,j} = \frac{n_{i,j-1} - 2n_{i,j} + n_{i,j+1}}{(\delta \tau)^2},
\]  

(3.85)
where \( n_{i,j} \) is the cell density of cells aged \([j\tau_s, (j+1)\tau_s)\) in the time interval \([it_s, (i+1)t_s)\) where \( t_s \) and \( \tau_s \) are the length of the discretised elements. This leads to the finite difference scheme

\[
n_{i+1,j} = n_{i,j} \left( 1 - \delta th_{i,j} + \frac{(\delta t)^2}{2} h_{i,j} \right) + \frac{n_{i,j+1} - n_{i,j-1}}{2\delta \tau} \left( -\delta t + (\delta t)^2 h_{i,j} \right) + \frac{n_{i,j-1} - 2n_{i,j} + n_{i,j+1}(\delta t)^2}{(\delta \tau)^2}.
\]

(3.86)

Which, on re-arranging becomes

\[
n_{i+1,j} = n_{i,j} \left( 1 - \frac{(\delta t)^2}{2(\delta \tau)^2} - \delta th_{i,j} + \frac{(\delta t)^2}{2} h_{i,j} \right) + n_{i,j+1} \left( \frac{(\delta t)^2}{2(\delta \tau)^2} + \frac{\delta t}{2\delta \tau} + \frac{(\delta t)^2}{2\delta \tau} h_{i,j} \right) + n_{i,j-1} \left( \frac{(\delta t)^2}{2(\delta \tau)^2} + \frac{\delta t}{2\delta \tau} - \frac{(\delta t)^2}{2\delta \tau} h_{i,j} \right).
\]

(3.87)

Because of the ‘dispersive’ nature of any numerical difference scheme if \( \delta \tau \neq \delta t \) additional errors are introduced at each time step. For example if at \( t = 0 \) all cells are age zero and the age step is set to \( \epsilon \) and the time step set to \( \frac{\epsilon}{2} \), then after evolving the system for one time step there would be cells whose age is \( \epsilon \), this clearly makes no sense. Similarly if the time step is set to \( 2\epsilon \) after one step there are no cells present whose age is \( 2\epsilon \) since \( \tau \leq \epsilon \) for all cells. Hence, additional interpolation is required if the age and time steps are not equal. By setting \( \delta t = \delta \tau = \alpha \) Equation (3.87) becomes

\[
n_{i+1,j} = n_{i,j} \left\{ \frac{\alpha^2}{2} - \alpha \right\} h_{i,j} + n_{i,j+1} \left( \frac{\alpha}{2} h_{i,j} \right) + n_{i,j-1} \left( 1 - \frac{\alpha}{2} h_{i,j} \right).
\]

(3.88)

### 3.4.1 Stability of the Numerical System

For a numerical scheme to produce accurate solutions to a partial differential equation, not only must the error at each time step be small enough, any errors must not grow exponentially, i.e. the numerical scheme must also be stable. If the nutrient supply is unlimited and uptake is uniform then the cell cycle may be simplified into two ‘phases’, \( G_{1b} \) on its own and the remaining phases all put together. A two compartment model is not suitable for analysing the dynamics of a population of cells as too much information is lost by combining the \( MAIN \) phase and \( G_{1a} \) phases of the model discussed in Section 3.2, in particular the timing of the cell division. However, a two compartment model is
sufficient for conducting a stability analysis. Once the system has reached steady growth (i.e. no further input from $G_1$) then it may be represented as shown in Figure 3.10 where $X$ and $Y$ represent the two ‘phases’. To perform the stability analysis the time step matrix is constructed, the norm of which is shown to be bounded. It is helpful to start by defining some notation.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{two_compartment_model.png}
\caption{Illustration of the Two Compartment Model Used in the Stability Analysis of the Numerical Scheme.}
\end{figure}

**Notation**

If the numerical scheme is discretised into elements of time of length $t_s$ and age elements of length $\tau_s$ then let cells in phase $X$ of age $\in [i\tau_s, (i+1)\tau_s)$ in the time interval $\in [jt_s, (j+1)t_s)$ be denoted by $X^i_j$. Also let all cells in phase $X$ in the time interval $\in [mt_s, (m+1)t_s]$ be denoted by $X_m$, where $X_m$ is now a column vector. Also assume the time line is moved such that at $t = t_0, t' = 0$, where $t'$ is the time used for the purposes of the subscript; for convenience the ' notation is now dropped.

**Construction of the Time Step Matrix**

Let the maximum durations of the $X$ and $Y$ phases be $N\tau_s$ and $K\tau_s$ respectively then at time $t = t_0$,

\begin{align*}
X^0_0 & \quad \text{cells entering } X, \\
X^{N-1}_0 & \quad \text{cells in } X \text{ dying due to old age at the next time step}, \\
Y^0_0 & \quad \text{cells entering } Y, \\
Y^{K-1}_0 & \quad \text{cells leaving } Y \text{ and doubling at the next time step}.
\end{align*}

(3.89)
Clearly,
\[ X_a^0 = 2Y_a^{K-1}. \] (3.90)

Also the cells entering \( Y \) are a function of the cells who were in \( X \) at the previous time step, therefore
\[ Y_a^0 = h(X_{a-1}), \] (3.91)
where \( h(v) \) is the probability of transition from \( X \) to \( Y \). Since nothing happens to the cells during their time in \( Y \), it can be thought of as merely a time delay phase, therefore
\[ Y_a^j = Y_{a-1}^{j-1} \text{ for } 1 \leq j < K. \] (3.92)

Note, the inequality is strictly less than \( K \) as cells of age \( K\tau_s \) have undergone division and the offspring are now in \( X_a^0 \).

Assuming a finite central difference scheme is used for calculating the cell densities in the \( X \) phase then
\[ X_a^i = f(X_{a-1}^{i-1}, X_{a-1}^i, X_{a-1}^{i+1}) \text{ for } 1 \leq i < N, \] (3.93)
and
\[ X_a^N = f(X_{a-1}^{N-1}, X_{a-1}^N). \] (3.94)

From equations (3.90) and (3.92) it is clear that
\[ X_a^0 = 2Y_a^{K-1} = 2Y_a^{K-2} = \ldots = 2Y_a^{0-K}. \] (3.95)

Now using equation (3.91) yields
\[ X_a^0 = 2h(X_a^{0-K-1}). \] (3.96)

Equations (3.90-3.96) may be expressed in matrix notation as
\[
\begin{bmatrix}
X_{m+1}^0 \\
X_{m+1}^{1-N-2} \\
X_{m+1}^{N-1} \\
Y_{m+1}^0 \\
Y_{m+1}^{1-K-1}
\end{bmatrix}
= M
\begin{bmatrix}
X_m^0 \\
X_m^{1-N-2} \\
X_m^{N-1} \\
Y_m^0 \\
Y_m^{1-K-1}
\end{bmatrix},
\] (3.97)

where \( M \) is an \((N + K) \times (N + K)\) matrix. Details of the construction of \( M \) may be found in Appendix B.
To prove the numerical scheme is stable it is sufficient to show \[58\] that the norm of \( \mathbf{M} \) in Equation (3.97) satisfies
\[
\| \mathbf{M} \| \leq 1 + \kappa \alpha, \tag{3.98}
\]
where \( \delta t = \delta \tau = \alpha \) and \( \kappa \) is a constant independent of \( \alpha \). In Appendix C the existence of \( \kappa \) is discussed. It can be shown that if the trapezium rule is used for approximating Equation (3.91) then the norm of \( \mathbf{M} \) is given by
\[
\| \mathbf{M} \| = \sup \{2, \alpha \sum_{j=1}^{N-2} h(j) + \frac{\alpha}{2} (h(0) + h(N - 2))\}. \tag{3.99}
\]
For the transition functions considered \( h \) is monotonically increasing so
\[
\alpha \sum_{j=1}^{N-2} h(j) + \frac{\alpha}{2} (h(0) + h(N - 2)) \leq \alpha \int_{0}^{X_{\tau \text{max}}} h(\tau) d\tau, \tag{3.100}
\]
it is therefore sufficient to show \( \alpha \int_{0}^{X_{\tau \text{max}}} h(\tau) d\tau \) remains bounded. For the sigmoidal transition rule
\[
\int_{0}^{X_{\tau \text{max}}} \frac{e^{(\tau - \theta \frac{\alpha}{2})}}{1 + e^{(\tau - \theta \frac{\alpha}{2})}} d\tau = \left[ \ln \left( 1 + e^{\tau - \theta \frac{\alpha}{2}} \right) \right]_{0}^{X_{\tau \text{max}}}, \tag{3.101}
\]
which for typical \( \theta \) values this is approximately equal to \( \ln \left( 1 + e^{X_{\tau \text{max}} - \theta \frac{\alpha}{2}} \right) \). For \( X_{\tau \text{max}} \leq \theta \frac{\alpha}{2} \) then
\[
\ln \left( 1 + e^{X_{\tau \text{max}} - \theta \frac{\alpha}{2}} \right) \approx e^{X_{\tau \text{max}} - \theta \frac{\alpha}{2}} \leq 1. \tag{3.102}
\]
For \( X_{\tau \text{max}} > \theta \frac{\alpha}{2} \) then
\[
\ln \left( 1 + e^{X_{\tau \text{max}} - \theta \frac{\alpha}{2}} \right) \approx X_{\tau \text{max}} - \theta \frac{\alpha}{2}. \tag{3.103}
\]
Thus, in all cases \( \| \mathbf{M} \| \) remains bounded. In most cases \( (X_{\tau \text{max}} - \theta \frac{\alpha}{2}) \) \( \alpha < 1 \), this leads to a stronger constraint on the bound i.e. \( \| \mathbf{M} \| \leq 2 \). An alternative approach for showing \( \| \mathbf{M} \| \) remains bounded is given in Appendix C.
3.5 PDE Model Validation and the Effect of The Transition Function

The new, three compartment age structured model has two fewer phases than the CelCyMUS model. Additionally, a new sigmoidal transition function has been implemented. To test the validity of the new model comparisons are needed with experimental data. Experimental data from [31] was chosen and concerns a batch experiment which was conducted using a mouse-mouse hybridoma cell line (mm321). This data was chosen as it allowed a direct comparison between the fitting of the three compartment model and the CelCyMUS model. This is discussed at the end of this section. In this experiment 28% of the starting cell population did not divide but remained viable, 36% of the starting population were evenly distributed in the S phase of the cell cycle and the remaining 36% were initially at the beginning of the $G_{1b}$ phase. For the purposes of modelling it was assumed the cells starting in the $G_{1b}$ phase were of a phase age between zero and two hours. The numerical scheme described in Section 3.2, was implemented using both sigmoidal and constant transition ($h = c$) functions. Parameters for the length of different phases were taken from [23], and are stated in Table 3.1. The $\theta$ and $c$ parameters were allowed to vary in the sigmoidal and constant transition rules respectively, until a best fit had been obtained. Several starting values for $\theta$ and $c$ were used in the optimisations of the fits to ensure the global best fits had been found for each transition rule and that the results were not a local minimum. Optimisations were carried out using Matlab’s [44] least squares curve fitting algorithm `lsqcurvefit`. The Matlab code for these optimisations is available from [15].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Notation</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum age in $G_{1a}$ phase</td>
<td>$T_{G1a}$</td>
<td>2.5 hours</td>
</tr>
<tr>
<td>Maximum age in $G_{1b}$ phase</td>
<td>$T_{G1b}$</td>
<td>10 hours</td>
</tr>
<tr>
<td>Maximum age in $S$ phase</td>
<td>$T_S$</td>
<td>5 hours</td>
</tr>
<tr>
<td>Maximum age in $G_2 + M$ phase</td>
<td>$T_{G2+M}$</td>
<td>4 hours</td>
</tr>
</tbody>
</table>

Table 3.1: Parameters from [23].
As can be seen in Figure 3.11, both the constant transition rule (Figure 3.11a) and the sigmoidal rule (Figure 3.11b) provide a good fit to the experimental data resulting in residual norm values of 0.1 and 0.2 respectively. The parameters in Table 3.1 were varied by ±20%. Different values for the Table 3.1 parameters resulted in different values for the fitted parameters ($\theta$ and $c$) values but did not significantly change the goodness of the fit shown in Figure 3.11 with no residual norms exceeding 0.2. Note that the model did not impose any restrictions on the available nutrients, indicating nutrients were not a limiting factor for cell growth over the course of the experiment. This suggests, that if population growth is the only concern, that a constant transition rule is sufficient.

No measure of goodness of fit was provided in [23], however visual inspection of Figure 2 in [23] indicates that the fit to the experimental data provided by the three compartment model is comparable to that of the CeICyMUS model.
3.5 PDE Model Validation and the Effect of The Transition Function

Figure 3.11: Growth Curves Produced by Using a Constant Transition Rule (a) and a Sigmoidal Transition Rule (b) Fitted Against Experimental Batch Data Presented in [23].
3.5.1 The Effect of the Transition Function

Although the effect of the different transition functions is not apparent in the fitting of the model to the experimental growth curve, the transition function does impact on the phase distribution of cells.

In the experimental data used to validate the model the initial population of cells was partially synchronised using a thymidine double block. Full details of this block can be found in [31]. This partial synchronisation meant the initial population of cells was situated in the $S$ phase and the latter part of the $G_1$ phase, i.e. the $G_{1b}$ compartment. It therefore seems reasonable to assume that most cells will initially progress round the cycle in a group. This grouping would result in the phase distribution being oscillatory. The oscillations would be expected to decay slowly as the synchronicity of the cell population was lost. Such oscillations may be one cause for apparent ‘errors’ in phase distributions obtained from such experiments as the timing of observations would need to occur at known positions on the oscillation, the period of which may not be known. The time scale required for the transient oscillations to have decayed sufficiently is of the order of 500 hours, at this point the population of cells is undergoing exponential growth and has a steady DNA distribution. Due to the length of time needed for this to occur and the number of data points required it is not feasible to obtain experimental data.

To fully appreciate the differences these transition functions have on the underlying model properties the percentages of cells in each compartment may be compared once transient oscillations have decayed and the system has reached a steady state of phase distributions. In order to investigate this, the mathematical model was numerically integrated using the same parameters and initial conditions used in Section 3.5 for long enough that a steady phase distribution had been obtained for both the sigmoidal and constant transition functions. The results are shown in Figure 3.12. The two resulting phase distributions differ in two important ways. Firstly, both simulations initially show an oscillation in the phase distribution, however the rate of decay of the oscillations depends on the transition function chosen, with the oscillations decaying much more slowly for a sigmoidal transition function. The difference in the decay rates may be appreciated by considering the area under the cumulative probability function $F$ for the different transition functions (Figures 3.13 and 3.14). For a steep sigmoidal probability distribution function the area under the curve initially increases slowly then has a rapid increase for a short time interval then
returns to a slow increase as shown in Figure 3.14. This rapid increase would result in the majority of the population remaining in a group as it progressed round the cycle, with each complete cycle the population would disperse slightly due to the ages corresponding to a low probability of transition. With the value of the constant transition function used in this simulation the area under the corresponding cumulative probability distribution function does not change as rapidly as with the sigmoidal function as shown in Figure 3.13. This results in the population of cells transitioning more evenly, leading to a more rapid de-synchronisation. The second important difference between the two simulations is that once the transient oscillations have decayed the percentages of cells in each of the model’s ‘phases’ differ: in the sigmoidal transition rule there are 20.2%, 33.3% and 46.5% in the $G_{1a}$, $G_{1b}$ and MAIN phases respectively, whereas in the constant transition rule these change to 22.6%, 24.4% and 53.0%.
Figure 3.12: Proportions of Cells in Each Phase Using a Constant Transition Rule (a) and a Signoidal Transition Rule (b).
Figure 3.13: Constant Transition Function (a) With the Corresponding Cumulative Probability of Transition (b) As a Function of $G_{1b}$ Age.
3.5 PDE Model Validation and the Effect of The Transition Function

Figure 3.14: Sigmoidal Transition Function (a) With the Corresponding Cumulative Probability of Transition (b) As a Function of \( G_{1b} \) Age.
It has been shown that an age-structured population balance model provides a good fit to a growth curve obtained experimentally. The model shows however there is a noticeable change in the proportion of cells in each phase for the two different transition functions considered. The sigmoidal transition function predicts 53.5\% of the cell population being in the $G_1$ phase, whilst the constant transition function places 47.0 \% of cells in the $G_1$ phase. For comparison the CelCyMUS model presented in [23] placed 53.0\% of the cell population in the $G_1$ phase.

Chemotherapy drugs can be divided into several types, each of which target a specific process within the cell cycle such as RNA synthesis or cell division. Hence the efficacy of many chemotherapy drugs (e.g. [11], [40] and [50]) is dependent on the cell cycle phase. The radiosensitivity of cells is also phase dependent (e.g. [13], [43] and [64]) with cells in the $M$ (mitotic) phase having their chromosomes arranged in a line prior to separation making them particularly sensitive to ionising radiation. Due to the phase dependent nature of chemotherapy drugs and radiotherapy, knowledge of how the cells progress through the different phases is crucial. Since the relationship between cell phase and efficacy may be non-linear a small difference in phase distribution may produce a large change in the efficacy of treatments resulting in the model producing results outside the bounds of experimental error. Therefore, the difference in the phase distributions produced by this model, using the different transition functions, will effect the model’s ability to accurately represent the effects of a given treatment on a population of cells. Consequently, it is important to ascertain the correct transition function if such models are to be used to give a quantitative prediction of the cell population’s response to treatments. Whilst there is no consensus on the error on cell phase distributions obtained using flow cytometry [18] the difference in phase distributions produced by the model with the different transition rules lie within the typical bounds of current experimental error ( [18], [19] and [36]). As noted in Section 3.5.1 the difficulty of measuring the phase distribution may be compounded by underlying oscillations induced by the blocking. Improvements in experimental techniques may reduce the level of potential error in phase distributions obtained experimentally, this may allow some transition functions to be discounted.

Thus, the form of the probability distribution function controlling the $G_1 - S$ checkpoint in an age structured population balance model has little impact on the models ability to fit to experimental data, due to the typical bounds of experimental error. The lack
of effect of the form of the probability transition function explains why the quadratic transition function used in [23] fitted experimental data despite having a singularity. As such, a simplified transition function may be used to gain a qualitative understanding of the dynamics of a population of cells.

3.6 Ordinary Differential Equation Model Validation and Comparison of Ordinary Differential Equation and Partial Differential Equation Models

As discussed in Section 2.1.2 it is possible to construct a structured ODE model for the cell cycle which may be adequate if only a limited amount of information about the population is required. To determine the suitability of an ODE model for estimating the total number of cells and the proportion of cells in each phase it is necessary to compare the results obtained with those from a PDE based model such as that described in Section 3.2. The PDE model described in Section 3.2 consists of three compartments \(G_{1a}, G_{1b}\) and \(MAIN\). Therefore, any ODE model need only distinguish between the \(G_1\) phase and the remainder of the cell cycle. Since the \(G_{1a}\) and \(G_{1b}\) division concerns the minimum amount of time a cell must spend in the \(G_1\) phase before proceeding through the cycle it is not possible to include this in an ODE framework as there is no distinction between cells based upon their age. As such a two compartment ODE model is suitable for this comparison. Consider a two compartment ODE model. One compartment \(X\), containing cells in the \(G_1\) phase of the cycle and the other compartment \(Y\) containing the remainder of the cell population, as illustrated in Figure 3.15. Let the rate at which cells progress from the \(X\) to the \(Y\) compartment occur at a constant rate \(a\) and the rate at which cells leave \(Y\) and divide so that two new cells join \(X\) occur at the constant rate \(b\), then the system may be described mathematically by two linear first order differential equations, one for each compartment as

\[
\frac{dX(t)}{dt} = 2bY(t) - aX(t),
\]

\[
\frac{dY(t)}{dt} = aX(t) - bY(t).
\]  

(3.104)

where \(X(t)\) and \(Y(t)\) are the number of cells in the two compartments at time \(t\). Equations (3.104) may be solved analytically if suitable initial or boundary conditions are known. To
allow a direct comparison of this ODE model with the PDE model described in Section 3.2 the ODE model may be fitted to the experimental data presented in [31]. Unlike the PDE model where the $\theta$ and $c$ values were allowed to vary the ODE model has two parameters, $a$ and $b$, which need to be optimised to fit the growth curve. Several starting values for $a$ and $b$ were used in the optimisation to ensure the global best fits had been found and that the results were not a local minimum. Optimisations were carried out using Matlab’s [44] least squares curve fitting algorithm lsqcurvefit. Unlike, with the PDE model, it was found that the ODE model was not very sensitive to the values of $a$ and $b$ and that different combinations of $a$ and $b$ provided good fits to the experimental data as shown in Figure 3.16. Several such sets of values and the resulting residual norms are given in Table 3.2 together with the fraction of cells in the $Y$ compartment which is analogous to the $MAIN$ compartment in the PDE model.

![Diagram of a Two Compartment ODE Model](image)

**Figure 3.15: Illustration of a Two Compartment ODE Model.**

<table>
<thead>
<tr>
<th>$a$</th>
<th>$b$</th>
<th>$Y$ Percentage</th>
<th>Residual Norm of Fit</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0791</td>
<td>0.1258</td>
<td>32.2</td>
<td>0.16</td>
</tr>
<tr>
<td>0.1138</td>
<td>0.0946</td>
<td>41.4</td>
<td>0.18</td>
</tr>
<tr>
<td>0.1266</td>
<td>0.0883</td>
<td>49.0</td>
<td>0.18</td>
</tr>
<tr>
<td>0.1316</td>
<td>0.0863</td>
<td>50.4</td>
<td>0.18</td>
</tr>
<tr>
<td>0.1342</td>
<td>0.0853</td>
<td>51.0</td>
<td>0.18</td>
</tr>
</tbody>
</table>

**Table 3.2: Parameters Fitted to A Two Compartment ODE Model.**
Figure 3.16: Growth Curve Produced from a Two Compartment ODE Model Fitted Against Experimental Batch Data Presented in [23].

By looking at the proportion of cells in the $Y$ compartment it can be seen that the bottom three rows in Table 3.2 are close to the values obtained using a three compartment PDE model cf. Figure 3.12. However, it should be noted that using the formulae given in Equations (2.35-2.38) the average time cells spends in the $X$ and $Y$ compartments is approximately $\frac{1}{a} \approx 7.5$ hours and $\frac{1}{b} \approx 11.5$ hours respectively. This is in disagreement with the experimental values given in Table 3.1. By carefully comparing the growth curves shown in Figure 3.11 and Figure 3.16 it can be seen that whilst the ODE model produces a good overall fit the growth curve is smooth and does not capture the step like nature of the experimental data, unlike the PDE model. This is due to the fact that the PDE model is age structured and can therefore take into account the initial age distribution of the population of cells.
3.7 Cell Cycle Model Summary

In this Chapter two new models were formulated. The first of these was a structured ODE model consisting of four compartments, each corresponding to a different phase of the cell cycle, details of which were given in Section 3.1. General results for the phase distribution of the population were derived. For the specific case where the rate constants were chosen to all be unity it was shown that the phase distribution of the population obtained using this model is identical to that given by Steel’s formulae. For the more general case the reasons why the phase distribution obtained using this method differs from Steel’s formulae were discussed.

Using the CelCyMUS model as a starting point a new three compartment, age-structured PDE model was presented in Section 3.2. Section 3.3 contains a comprehensive review of the transition functions which model how the cells progress from the $G_1$ to $S$ phase of the cycle. Existing constant [6], quadratic [22] and [23], and sigmoidal [57] transition functions were discussed in detail. It was noted that the constant form of the transition function is independent of the time spent in the $G_{1b}$ phase and is therefore not biologically realistic as it implies all cells in the $G_{1b}$ phase have an equal probability of progressing to the $S$ phase regardless of how long they have spent acquiring nutrients and preparing for DNA synthesis. Despite this it was shown in Section 3.5.1 that the use of such a transition function may be justified. The mathematical validity of the quadratic transition function was called into question due to the possible singularity which can occur. This transition function was therefore deemed unsuitable. The sigmoidal transition function proposed by [57] was considered but it was noted that the sigmoidal form suggested has multiple independent parameters when non-dimensionalised, making fitting of the model to experimental data more difficult. Motivated, by the biologically realistic nature of the sigmoidal transition function given in [57] a new sigmoidal transition function was presented together with the appropriate non-dimensionalisation consistent with the non-dimensionalisation given in Section 3.2 for the new simplified age-structured model. This new sigmoidal transition function has only one independent parameter which needs to be fitted, thus making the problem more tractable.

In Section 3.4 a numerical scheme for the new simplified age-structured model was presented, together with a discussion on the scheme’s stability. It was shown that for a monotonically increasing probability of transition the numerical scheme is stable.
Section 3.5 saw the simplified age structured model with both constant and sigmoidal transition functions compared with experimental data. The experimental data chosen concerned a batch experiment which was conducted using a mouse-mouse hybridoma cell line (mm321) [31]. It was shown that if population growth is the only concern, that a constant transition rule is sufficient to accurately fit the experimental data. However, if the phase distribution for the cell population is important then the form of the transition function becomes more critical. Further discussion on this may be found in [16].

The ODE model framework discussed in Section 3.1 was modified to allow direct comparison with the PDE model described in Section 3.2. The ODE model was then fitted to the same experimental data as that used in Section 3.5 and the ODE and PDE results were compared. All of the parameter values obtained in the ODE optimisation provided a good match to the experimental cell growth curve. The fraction of cells in the different compartments varies by 19% whilst lying outside the bounds of experimental error ([18], [19] and [36]) this is still sufficiently accurate to gain a qualitative understanding of the behaviour of the cell population.

From this comparison it may be concluded that if the duration of the phases is not important and the only information required is a qualitative understanding of the phase distribution or an approximation on the total number of cells within the population then a simple ODE model may be used. If a more accurate measure of the phase distribution of the cell population is required then a PDE or integral equation model may be required.
Chapter 4

Integral Equations for an Age Structured Population Balance Model

Systems of PDEs governing the cell population density may also be expressed in terms of integral equations. By converting a system of PDEs to integral equations it may be possible to utilise different analytical techniques to gain a greater understanding of the system. There may also be computational advantages to using this approach. Much of the analysis in this chapter is motivated by, and closely follows, work undertaken in [55]. In this chapter a four compartment age-structured model, similar to that discussed in Section 3.4, is constructed and the corresponding differential equations for the population density in each phase are found. Two new functions $M(t)$ and $P(t)$ are introduced, where $M(t)$ represents the number of cells dividing and $P(t)$ is the total number of cells in the population. The system is then expressed in terms of integral equations for these two new functions. Once $M(t)$ and $P(t)$ have been found the integral equations for the population densities are readily derived by solving the partial differential equations along characteristics. It should be noted in this chapter the integral equations are derived and no analysis of the resulting system is undertaken. The integral equation approach has been included to indicate alternative methods for the modelling of the cell population are available.
4.1 Construction of the Population Balance Model

Consider a cell cycle model comprising of four compartments. The first of these consists of the first part of the $G_1$ phase, $G_{1a}$, denoted in this chapter by $A$. The second part $B$, contains the latter part of the $G_1$ phase, $G_{1b}$. The $S$, $G_2$ and $M$ phases are all contained in the $C$ compartment. The final compartment contains non-cycling cells which have not entered $C$ after reaching the maximum time allowed in the $B$ compartment, the compartment containing these cells is denoted $D$. It should be noted that this model is the same as that described in Section 3.2 with the addition of compartment $D$. In [55] a further compartment is added which includes cells which do not divide at the end of $C$ due to some overcrowding function $f(P)$; this scenario is not considered here. The model presented here may be extended to allow some of the population in $C$ to re-enter the main cycling population at a later stage when the conditions for progression, i.e. nutrient availability, improve. A graphical representation of this model is shown in Figure 4.1.

For $i \in \{A, B, C, D\}$, let $n_i(t, \tau_i)$ denote the population density of cells in the compartment $i$ at time $t$ and of age $\tau_i$. For ease, the compartment subscript on $\tau$ will be omitted throughout this chapter, unless it is ambiguous which $\tau_i$ is being referred to. Let $T_i$ be the maximum duration cells may spend in compartment $i$. $T_A$, $T_B$ and $T_C$ are assumed
to be finite, while $T_D$ is infinite. In reality $T_D$ is also finite as the non-cycling cells will eventually die then degrade, however for the purposes of this model it is sufficient to assume that it is infinite.

Assuming there is no exit from the system then for $i \in \{A, C, D\}$, $n_i(t, \tau)$ satisfies the PDEs

$$\frac{\partial n_i(t, \tau)}{\partial t} + \frac{\partial n_i(t, \tau)}{\partial \tau} = 0 \quad \tau \in (0, T_i), \ t \geq 0, \quad (4.1)$$

and

$$\frac{\partial n_B(t, \tau)}{\partial t} + \frac{\partial n_B(t, \tau)}{\partial \tau} = -h(\tau)n_B(t, \tau), \quad \tau \in (0, T_B), \ t \geq 0, \quad (4.2)$$

where $h(\tau) \in C([0, T_B]; [0, 1])$ is a transition function, as described in Section 3.3, which describes the proportion of cells which leave the $B$ compartment and enter the $C$ compartment at age $\tau$.

The initial conditions for the system are

$$n_i(0, \tau) = 0 \quad \tau \in (0, T_i), \quad (4.3)$$

for $i \in \{A, B, C, D\}$.

The total number of the cells, both cycling and quiescent, is given by $P(t)$, where

$$P(t) = \sum_i \int_0^{T_i} n_i(t, \tau) \, d\tau. \quad (4.4)$$

This is a special case of the $P(t)$ defined in [55]. Here $P(t)$ is defined as

$$P(t) = \sum_i \int_0^{T_i} w_i(\tau)n_i(t, \tau) \, d\tau, \quad (4.5)$$

where $w_i(\tau)$ is a weighting function which allows cell degradation of the non-cycling cells in the $D$ compartment to be considered. This is necessary if there is an overcrowding function that is dependent on $P(t)$. Since the model considered here does not include any form of overcrowding this is not needed.

To determine the boundary conditions at $\tau_i = 0$ for $i \in \{A, B, C, D\}$ it is first necessary to define $M(t)$. Let

$$M(t) = n_C(t, T_C), \quad (4.6)$$
4.2 Solution of the PDE System

Using the method of characteristics it is possible to express the solution for the system of PDEs in terms of integrals involving $M(t)$. It is then possible to obtain an expression for $P(t)$. In order to solve the system of PDEs the relative maximum durations of the different compartments are required. By considering the maximum duration of the different phases in a mouse-mouse hybridoma cell line (mm321) [31] it may be assumed $T_B > T_C > T_A$ and that $T_A + T_C > T_B$. For different cell lines this may not be the case but the methodology described in this section will work for different relative phase age assumptions.

4.2.1 Solution for Compartment $A$

Using the method of characteristics the solution of the equation for the $A$ compartment is given by

$$n_A(t, \tau) = \begin{cases} 
    n_A(0, \tau - t) & \text{if } t < \tau, \\
    n_A(t - \tau, 0) & \text{if } t \geq \tau,
  \end{cases}$$

$$= \begin{cases} 
    0 & \text{if } t < \tau, \\
    2M(t - \tau) & \text{if } t \geq \tau.
  \end{cases}$$

where $\psi(t)$ is a function which describes how cells enter the system from an initial quiescent phase, $G^s$. 

cf. in [55] $M(t) = f(P)n_C(t, T_C)$ where $f : \mathbb{R}_+ \to [0, 1]$ is a function such that $f(P)$ describes the proportion of cells which undergo division at the end of the $C$ compartment. 

The boundary conditions at $\tau = 0$ for $i \in \{A, B, C, D\}$ are given by

$$n_A(t, 0) = 2M(t),$$

$$n_B(t, 0) = n_A(t, T_A) + \psi(t),$$

$$n_C(t, 0) = \int_0^{T_B} h(\tau)n_B(t, \tau) d\tau,$$

$$n_D(t, 0) = n_B(t, T_B),$$

where $\psi(t)$ is a function which describes how cells enter the system from an initial quiescent phase, $G^s$. 

4.2 Solution of the PDE System
4.2 Solution of the PDE System

4.2.2 Solution for Compartment B

The solution of the equation for the B compartment is given by

\[ n_B(t, \tau) = \begin{cases} 
  n_B(0, \tau - t)e^{-\int_0^{\tau - t}h(s)\,ds} & \text{if } t < \tau, \\
  n_B(t - \tau, 0)e^{-\int_0^0 h(s)\,ds} & \text{if } t \geq \tau.
\end{cases} \tag{4.9} \]

However, from the initial condition given in Equation (4.3) it is clear for \( t < \tau \) that \( n_B(t, \tau) = 0 \). Substituting the solution for \( n_A(t, \tau) \) into Equation (4.7b) gives

\[ n_B(t, 0) = \begin{cases} 
  \psi(t) & \text{if } t < T_A, \\
  2M(t - T_A) + \psi(t) & \text{if } t \geq T_A.
\end{cases} \tag{4.10} \]

as the boundary condition at \( \tau = 0 \) for the B compartment. Using these boundary conditions, together with \( n_B(t, \tau) = 0 \) for \( t < \tau \), Equation (4.9) now becomes

\[ n_B(t, \tau) = \begin{cases} 
  0 & \text{if } t < \tau, \\
  \psi(t - \tau)e^{-\int_0^{\psi(t)} h(s)\,ds} & \text{if } \tau \leq t < \tau + T_A, \\
  \{2M(t - \tau - T_A) + \psi(t - \tau)\}e^{-\int_0^{\psi(t)} h(s)\,ds} & \text{if } t \geq \tau + T_A.
\end{cases} \tag{4.11} \]

4.2.3 Solution for Compartment C

The solution of the equation for the C compartment is given by

\[ n_C(t, \tau) = \begin{cases} 
  n_C(0, \tau - t) & \text{if } t < \tau, \\
  n_C(t - \tau, 0) & \text{if } t \geq \tau.
\end{cases} \tag{4.12} \]

From the initial condition given in Equation (4.3) it is clear \( n_C(t, \tau) = 0 \) for \( t < \tau \). To obtain the solution for \( t \geq \tau \) it is necessary to use the boundary condition given in Equation (4.7c).

For \( t < T_A \) the initial assumptions made about the relationships between the maximum compartment times mean \( t < T_B \). Therefore \( t < T_A \Rightarrow t < T_A + \tau \). Thus for \( 0 \leq t < T_A \),

\[ n_C(t, 0) = \int_0^{T_B} h(\tau)n_B(t, \tau)\,d\tau, \]

\[ = \int_0^t h(\tau)n_B(t, \tau)\,d\tau, \tag{4.13} \]

\[ = \int_0^t h(\tau)\psi(t - \tau)e^{-\int_0^{\psi(t)} h(s)\,ds}\,d\tau. \]
Let $\hat{\tau} = t - \tau$, then
\[
\int_0^t d\tau = \int_0^{\hat{\tau}} d\tau.
\]
This change of variables is applied to Equation (4.13), which, after dropping the tilde notation, becomes
\[
n_C(t, 0) = \int_0^t h(t - \tau)\psi(\tau)e^{-\int_0^{\tau} h(s) ds} d\tau \quad \text{if } 0 \leq t < T_A.
\] (4.14)

For $T_A \leq t < T_B$
\[
n_C(t, 0) = \int_0^{T_A} h(\tau)n_B(t, \tau) d\tau,
\]
\[
= \int_0^T h(\tau)n_B(t, \tau) d\tau.
\] (4.15)

Unlike the case $t < T_A$, $t$ may now be $< \tau + T_A$ or $\geq \tau + T_A$ depending on the value of $\tau$. Therefore to calculate the integral given by Equation (4.15) it must be split into the two cases $t - T_A < \tau$ and $t - T_A \geq \tau$, i.e.
\[
n_C(t, 0) = \underbrace{\int_0^{t-T_A} h(\tau)n_B(t, \tau) d\tau}_{\text{part (i)}} + \underbrace{\int_{T_A}^t h(\tau)n_B(t, \tau) d\tau}_{\text{part (ii)}}.
\] (4.16)

For part (i) $t \geq \tau + T_A$, therefore from Equation (4.11) it follows
\[
n_B(t, \tau) = \{2M(t - \tau - T_A) + \psi(t - \tau)\} e^{-\int_0^{\tau} h(s) ds}.
\] (4.17)

For part (ii) $t < \tau + T_A$ therefore,
\[
n_B(t, \tau) = \psi(t - \tau)e^{-\int_0^{\tau} h(s) ds}.
\] (4.18)

Substituting the expressions for $n_B(t, \tau)$ given in Equations (4.17-4.18) into Equation (4.16) gives
\[
n_C(t, 0) = \int_0^{t-T_A} h(\tau)\{2M(t - \tau - T_A) + \psi(t - \tau)\} e^{-\int_0^{\tau} h(s) ds} d\tau \quad \text{part (i)}
\]
\[
+ \int_{t-T_A}^t h(\tau)\psi(t - \tau)e^{-\int_0^{\tau} h(s) ds} d\tau \quad \text{part (ii)}.
\] (4.19)
Define $\hat{\tau} = t - \tau$ and $\check{\tau} = t - \tau - T_A$, then

$$\int_0^{t-T_A} d\tau = \int_0^{t-T_A} d\check{\tau},$$

and

$$\int_{t-T_A}^t d\tau = \int_0^{T_A} d\check{\tau}.$$  

Using these substitutions Equation (4.19) may now be written as

\begin{equation}
\begin{split}
n_C(t, 0) &= \int_0^{t-T_A} h(t - \hat{\tau} - T_A) \{2M(\hat{\tau}) + \psi(\hat{\tau} + T_A)\} e^{-\int_0^{\hat{\tau}} \psi h(s) ds} d\hat{\tau} \\
&\quad + \int_0^{T_A} h(t - \hat{\tau}) \psi(\hat{\tau}) e^{-\int_0^{\hat{\tau}} \psi h(s) ds} d\hat{\tau},
\end{split}
\end{equation}

\begin{equation}
\begin{split}
= \int_0^{t-T_A} 2h(t - \hat{\tau} - T_A) M(\hat{\tau}) e^{-\int_0^{\hat{\tau}} \psi h(s) ds} d\hat{\tau} \\
&\quad + \int_0^{T_A} h(t - \hat{\tau}) \psi(\hat{\tau}) e^{-\int_0^{\hat{\tau}} \psi h(s) ds} d\hat{\tau}.
\end{split}
\end{equation}

but $\check{\tau} = \hat{\tau} + T_A$, therefore the second of integrals in Equation (4.20) may be written as

\begin{equation}
\begin{split}
\int_{T_A}^t h(t - \hat{\tau}) \psi(\hat{\tau}) e^{-\int_0^{\hat{\tau}} \psi h(s) ds} d\hat{\tau}.
\end{split}
\end{equation}

Thus, Equation (4.20) becomes

\begin{equation}
\begin{split}
n_C(t, 0) &= \int_0^{t-T_A} 2h(t - \hat{\tau} - T_A) M(\hat{\tau}) e^{-\int_0^{\hat{\tau}} \psi h(s) ds} d\hat{\tau} \\
&\quad + \int_0^t h(t - \hat{\tau}) \psi(\hat{\tau}) e^{-\int_0^{\hat{\tau}} \psi h(s) ds} d\hat{\tau}.
\end{split}
\end{equation}
Proceeding in a similar manner for $T_B < t < T_A + T_B$ gives

\[
n_C(t, 0) = \int_0^{T_B} h(\tau)n_B(t, \tau) \, d\tau, \\
= \int_0^{t-T_A} h(\tau)n_B(t, \tau) \, d\tau \left[ \int_0^{T_B} h(\tau)n_B(t, \tau) \, d\tau \right] \text{ part (i)} \\
+ \int_{t-T_A}^{T_B} h(\tau)n_B(t, \tau) \, d\tau \text{ part (ii)} \\
= \int_0^{t-T_A} h(\tau)\left\{ 2M(t - \tau - T_A) + \psi(t - \tau) \right\} e^{-\int_0^\tau h(s) \, ds} \, d\tau \text{ part (i)} \\
+ \int_{t-T_A}^{T_B} h(\tau)\psi(t - \tau)e^{-\int_0^\tau h(s) \, ds} \, d\tau. \\
\tag{4.23}
\]

Making the substitutions $\hat{\tau} = t - \tau - T_A$ and $\check{\tau} = t - \tau$ into parts (i) and (ii) respectively leads to

\[
n_C(t, 0) = \int_0^{t-T_A} h(t - \hat{\tau} - T_A)\left\{ 2M(\hat{\tau}) + \psi(\hat{\tau} + T_A) \right\} e^{-\int_{\hat{\tau}}^{\tau} T_A h(s) \, ds} \, d\hat{\tau} \\
+ \int_{t-T_A}^{T_A} h(t - \check{\tau})\psi(\check{\tau})e^{-\int_0^{\check{\tau}} T_A h(s) \, ds} \, d\check{\tau}, \\
= \int_0^{t-T_A} 2h(t - \hat{\tau} - T_A)M(\hat{\tau})e^{-\int_{\hat{\tau}}^{\tau} T_A h(s) \, ds} \, d\hat{\tau} \\
+ \int_0^{t-T_A} h(t - \hat{\tau} - T_A)\psi(\hat{\tau} + T_A)e^{-\int_{\hat{\tau}}^{\tau} T_A h(s) \, ds} \, d\hat{\tau} \\
+ \int_{t-T_A}^{T_A} h(t - \check{\tau})\psi(\check{\tau})e^{-\int_0^{\check{\tau}} T_A h(s) \, ds} \, d\check{\tau}, \\
\tag{4.24}
\]
For \( t \geq T_A + T_B \) then

\[
\begin{align*}
n_C(t, 0) &= \int_0^{t-T_B} h(\tau) n_B(t, \tau) \, d\tau, \\
&= \int_0^{t-T_B} h(\tau) \{2M(t - \tau - T_A) + \psi(t - \tau)\} e^{-\int_0^\tau h(s) \, ds} \, d\tau, \\
&= \int_0^{t-T_B} h(\tau)2M(t - \tau - T_A)e^{-\int_0^\tau h(s) \, ds} \, d\tau \\
&\quad + \int_0^{t-T_B} h(\tau)\psi(t - \tau)e^{-\int_0^\tau h(s) \, ds} \, d\tau,
\end{align*}
\]

which upon making the substitutions \( \tilde{\tau} = t - \tau - T_A \) and \( \hat{\tau} = t - \tau \) becomes

\[
\begin{align*}
n_C(t, 0) &= \int_{t-T_A-T_B}^{t-T_A} 2h(t - \tilde{\tau} - T_A)M(\tilde{\tau})e^{-\int_0^{\tilde{\tau}} h(s) \, ds} \, d\tilde{\tau} \\
&\quad + \int_{t-T_B}^t h(t - \hat{\tau})\psi(\hat{\tau})e^{-\int_0^{\hat{\tau}} h(s) \, ds} \, d\hat{\tau}.
\end{align*}
\]

Thus, the solution for the \( C \) compartment given in Equation (4.12) together with the boundary conditions given in Equations (4.14), (4.22), (4.25) and (4.26), leads to

\[
n_C(t, \tau) = \begin{cases} 
0 & \text{if } t < \tau, \\
n_C(t - \tau, 0) & \text{if } t \geq \tau,
\end{cases}
\]

\[
= \begin{cases} 
0 & \text{if } t < \tau, \\
\int_0^{t-\tau} h(t - \tau - \sigma)\psi(\sigma)e^{-\int_0^{\sigma} h(s) \, ds} \, d\sigma & \text{if } \tau \leq t < T_A + \tau, \\
\int_0^{t-\tau-T_A} 2h(t - \tau - \sigma - T_A)M(\sigma)e^{-\int_0^{\tau+\sigma} h(s) \, ds} \, d\sigma \\
\quad + \int_0^{t-\tau} h(t - \tau - \sigma)\psi(\sigma)e^{-\int_0^{\tau+\sigma} h(s) \, ds} \, d\sigma & \text{if } T_A + \tau \leq t < T_B + \tau, \\
\int_0^{t-\tau-T_A} 2h(t - \tau - \sigma - T_A)M(\sigma)e^{-\int_0^{\tau+\sigma} h(s) \, ds} \, d\sigma \\
\quad + \int_{t-T_B}^{t-\tau} h(t - \tau - \sigma)\psi(\sigma)e^{-\int_0^{\tau+\sigma} h(s) \, ds} \, d\sigma & \text{if } T_B + \tau \leq t < T_A + T_B + \tau, \\
\int_0^{t-\tau-T_A-T_B} 2h(t - \tau - \sigma - T_A)M(\sigma)e^{-\int_0^{\tau+\sigma} h(s) \, ds} \, d\sigma \\
\quad + \int_{t-T_B}^{t-\tau} h(t - \tau - \sigma)\psi(\sigma)e^{-\int_0^{\tau+\sigma} h(s) \, ds} \, d\sigma & \text{if } t \geq T_A + T_B + \tau.
\end{cases}
\]

This result is analogous to the result presented in [55].
4.2.4 Solution for the $D$ Compartment

The solution for the $D$ compartment is given by

$$n_D(t, \tau) = \begin{cases} 0 & \text{if } t < \tau, \\ n_D(0, \tau-t) & \text{if } t \geq \tau. \end{cases}$$ (4.28)

From the initial condition given in Equation (4.3) it is clear $n_D(t, \tau) = 0$ for $t < \tau$. To obtain the solution for $t \geq \tau$ it is necessary to use the boundary condition given in Equation (4.7d). Substituting the solution given for $n_B(t, \tau)$ into Equation (4.7d) gives

$$n_D(t, 0) = \begin{cases} 0 & \text{if } t < T_B, \\ \psi(t - T_B) e^{-\int_0^{T_B} h(s) \, ds} & \text{if } T_B \leq t < T_A + T_B, \\ \{2M(t - T_A - T_B) + \psi(t - T_B)\} e^{-\int_0^{T_B} h(s) \, ds} & \text{if } t \geq T_A + T_B. \end{cases}$$ (4.29)

as the boundary condition at $\tau = 0$ for the $D$ compartment. Thus, the solution for the $D$ compartment is given by

$$n_D(t, \tau) = \begin{cases} 0 & \text{if } t < \tau, \\ n_D(t - \tau, 0) & \text{if } t \geq \tau. \end{cases}$$

$$= \begin{cases} 0 & \text{if } t < T_B + \tau, \\ \psi(t - \tau - T_B) e^{-\int_0^{T_B} h(s) \, ds} & \text{if } T_B + \tau \leq t < T_A + T_B + \tau, \\ \{2M(t - \tau - T_A - T_B) + \psi(t - \tau - T_B)\} e^{-\int_0^{T_B} h(s) \, ds} & \text{if } t \geq T_A + T_B + \tau. \end{cases}$$ (4.30)

4.3 Equations for $M(t)$

By the definition given in Equation (4.6), $M(t) = n_C(t, T_C)$. Using the solution of $n_C(t, \tau)$ given by Equations (4.27) it can be seen there are the following cases for $M(t)$.

If $t < T_C$ then

$$M(t) = 0.$$ (4.31)

If $T_C \leq t < T_A + T_C$ then

$$M(t) = \int_0^{t-T_C} h(t - T_C - \sigma) \psi(\sigma) e^{-\int_0^{t-T_C-\sigma} h(s) \, ds} \, d\sigma.$$ (4.32)
If $T_A + T_C \leq t < T_B + T_C$ then

$$M(t) = \int_{0}^{t-T_A-T_C} 2h(t - \sigma - T_A - T_C)M(\sigma)e^{-\int_{0}^{\sigma-T_A-T_C} h(s)\,ds} d\sigma$$

$$+ \int_{0}^{t-T_C} h(t - T_C - \sigma)\psi(\sigma)e^{-\int_{0}^{\sigma-T_C} h(s)\,ds} d\sigma. \quad (4.33)$$

However, since the range for $\sigma$ in the first integral is $\sigma \in \{0, t - T_A - T_C\}$, then $\sigma \leq T_B - T_A \forall t \in [T_C, T_A + T_C]$. The assumption $T_C \geq T_B - T_A$ means $\sigma \leq T_C \forall t \in [T_C, T_A + T_C]$. Using Equation (4.31) it is clear $M(\sigma) = 0$, thus the first of these integrals must also be zero. Therefore, for $T_A + T_C \leq t < T_B + T_C$

$$M(t) = \int_{0}^{t-T_C} h(t - T_C - \sigma)\psi(\sigma)e^{-\int_{0}^{\sigma-T_C} h(s)\,ds} d\sigma. \quad (4.34)$$

If $T_B + T_C \leq t < T_A + T_B + T_C$ then

$$M(t) = \int_{0}^{t-T_A-T_C} 2h(t - \sigma - T_A - T_C)M(\sigma)e^{-\int_{0}^{\sigma-T_A-T_C} h(s)\,ds} d\sigma$$

$$+ \int_{t-T_B-T_C}^{t-T_C} h(t - T_C - \sigma)\psi(\sigma)e^{-\int_{0}^{\sigma-T_C} h(s)\,ds} d\sigma. \quad (4.35)$$

Unlike the previous case the first of these integrals is not always zero since $T_B \geq T_C$. Because $M(\sigma) = 0$ for $\sigma < T_C$ the lower limit of the first of these integrals may be changed to $T_C$. For the integral to be strictly positive (this must be the case as it is a measure of cell density) the upper limit needs to be greater than the lower limit, i.e. $t - T_A - T_C \geq T_C$. It is possible for this inequality not to hold as the minimum value of $t - T_A - T_C$ is $T_B - T_A$ and by assumption $T_B - T_A \leq T_C$. Thus the upper limit is now $\max\{T_C, t - T_A - T_C\}$. Hence for $T_B + T_C \leq t < T_A + T_B + T_C$

$$M(t) = \int_{T_C}^{\max\{T_C, t-T_A-T_C\}} 2h(t - \sigma - T_A - T_C)M(\sigma)e^{-\int_{0}^{\sigma-T_A-T_C} h(s)\,ds} d\sigma$$

$$+ \int_{t-T_B-T_C}^{t-T_C} h(t - T_C - \sigma)\psi(\sigma)e^{-\int_{0}^{\sigma-T_C} h(s)\,ds} d\sigma. \quad (4.36)$$

If $t \geq T_A + T_B + T_C$ then

$$M(t) = \int_{t-T_A-T_C}^{t-T_C} 2h(t - \sigma - T_A - T_C)M(\sigma)e^{-\int_{0}^{\sigma-T_A-T_C} h(s)\,ds} d\sigma$$

$$+ \int_{t-T_B-T_C}^{t-T_C} h(t - T_C - \sigma)\psi(\sigma)e^{-\int_{0}^{\sigma-T_C} h(s)\,ds} d\sigma. \quad (4.37)$$
4.4 Equations for $P(t)$

In the same manner as before the lower limit on the first integral may be changed to
$max \{ T_C, t - T_A - T_B - T_C \}$, thus for $t \geq T_A + T_B + T_C$

$$M(t) = \int_{\max(T_C,t-T_A-T_B-T_C)}^{t-T_A-T_C} 2h(t - \sigma - T_A - T_C)M(\sigma)e^{-\int_{\sigma}^{t} - T_A - T_C h(s) ds } d\sigma$$

$$+ \int_{t-T_B-T_C}^{t-T_C} h(t - T_C - \sigma)\psi(\sigma)e^{-\int_{\sigma}^{t} - T_C h(s) ds } d\sigma.$$  \hfill (4.38)

4.4 Equations for $P(t)$

As defined in Equation (4.5)

$$P(t) = \sum_i P_i(t),$$

$$= \sum_i \int_0^{T_i} n_i(t, \tau) d\tau, \quad \text{for } i \in \{A, B, C, D\}. \hfill (4.39)$$

Using this, the integral equations for $P_i(t)$ are now derived.

4.4.1 Equations for $P_A(t)$

By definition,

$$P_A(t) = \int_0^{T_A} n_A(t, \tau) d\tau.$$

If $t < T_A$ the initial condition given in Equation (4.3) implies $t \geq \tau$, therefore the upper limit of the integral may now be changed to $t$. Furthermore, from Equation (4.8), $n_A(t, \tau) = 2M(t - \tau)$ for $t \geq \tau$, thus for $t < T_A$

$$P_A(t) = \int_0^{t} 2M(t - \tau) d\tau. \hfill (4.40)$$

Similarly, for $t \geq T_A$

$$P_A(t) = \int_0^{T_A} 2M(t - \tau) d\tau. \hfill (4.41)$$

For both Equations (4.40) and (4.41) the change of variables $\tilde{\tau} = t - \tau$ may now be applied, which, after dropping the tilde notation, gives

$$P_A(t) = \begin{cases} \int_0^{t} 2M(\tau) d\tau & \text{if } t < T_A, \\ \int_0^{T_A} 2M(\tau) d\tau & \text{if } t \geq T_A. \end{cases} \hfill (4.42)$$
4.4 Equations for $P_B(t)$

By definition,

$$P_B(t) = \int_0^{T_B} n_B(t, \tau) \, d\tau.$$  

If $t < T_A$ then $t < T_B$, using this together with the initial condition given in Equation (4.3) implies $t \geq \tau$, therefore the upper limit of the integral may now be changed to $t$. Furthermore, if $t < T_A$ then $t < T_A + \tau \quad \forall \tau$ so from Equation (4.11), $n_B(t, \tau) = \psi(t - \tau)e^{-\int_0^{\tau} h(s) \, ds}$.

Thus for $t < T_A$

$$P_B(t) = \int_0^t \psi(t - \tau)e^{-\int_0^{\tau} h(s) \, ds} \, d\tau.$$  \hspace{1cm} (4.43)

The change of variables $\hat{\tau} = t - \tau$ may now be applied to Equation (4.43), which after dropping the tilde notation, becomes

$$P_B(t) = \int_0^t \psi(\tau)e^{-\int_0^{\tau} h(s) \, ds} \, d\tau.$$  \hspace{1cm} (4.44)

If $T_A \leq t < T_B$ then, like for the case $t < T_A$, the upper limit of the integral may be changed to $t$. However, $t < T_A + \tau \quad \forall t \in [T_A, T_B]$ and $\tau \in [0, t)$, therefore the integral needs to be considered in the two parts

$$P_B(t) = \int_0^{t-T_A} n_B(t, \tau) \, d\tau + \int_{t-T_A}^t n_B(t, \tau) \, d\tau.$$  \hspace{1cm} (4.45)

For part (i) $t \geq \tau + T_A$, so from Equation (4.11), $n_B(t, \tau) = \{2M(t - \tau - T_A) + \psi(t - \tau)\} e^{-\int_0^{\tau} h(s) \, ds}$.

Similarly, for part (ii) $t < \tau + T_A$ so $n_B(t, \tau) = \psi(t - \tau)e^{-\int_0^{\tau} h(s) \, ds}$, thus

$$P_B(t) = \int_0^{t-T_A} \{2M(t - \tau - T_A) + \psi(t - \tau)\} e^{-\int_0^{\tau} h(s) \, ds} \, d\tau + \int_{t-T_A}^t \psi(t - \tau)e^{-\int_0^{\tau} h(s) \, ds} \, d\tau,$$

$$= \int_0^{t-T_A} 2M(t - \tau - T_A)e^{-\int_0^{\tau} h(s) \, ds} \, d\tau + \int_{t-T_A}^t \psi(t - \tau)e^{-\int_0^{\tau} h(s) \, ds} \, d\tau.$$  \hspace{1cm} (4.46)

The change of variables $\hat{\tau} = t - \tau - T_A$ and $\hat{\tau} = t - \tau$ may now be applied to the first and second of these integrals respectively which, after dropping the hat and tilde notations, gives

$$P_B(t) = \int_0^{t-T_A} 2M(\tau)e^{-\int_0^{\tau-T_A-\tau} h(s) \, ds} \, d\tau + \int_{t-T_A}^t \psi(\tau)e^{-\int_0^{\tau-T_A-\tau} h(s) \, ds} \, d\tau.$$  \hspace{1cm} (4.47)
If \( T_B \leq t < T_A + T_B \) the two cases \( t < T_A + \tau \) and \( t \geq T_A + \tau \) are still both possible so proceeding in a similar manner as for the \( T_A \leq t < T_B \) case gives

\[
P_B(t) = \int_{0}^{t-T_A} n_B(t, \tau) \, d\tau + \int_{t-T_A}^{T_B} n_B(t, \tau) \, d\tau,
\]

\[
= \int_{0}^{t-T_A} \{2M(t - \tau - T_A) + \psi(t - \tau)\} e^{-\int_{\tau}^{t} h(s) \, ds} \, d\tau + \int_{t-T_A}^{T_B} \psi(t - \tau) e^{-\int_{\tau}^{t} h(s) \, ds} \, d\tau,
\]

\[
= \int_{0}^{t-T_A} 2M(t - \tau - T_A) e^{-\int_{\tau}^{t} h(s) \, ds} \, d\tau + \int_{t-T_A}^{T_B} \psi(t - \tau) e^{-\int_{\tau}^{t} h(s) \, ds} \, d\tau,
\]

\[
= \int_{0}^{t-T_A} 2M(\tau) e^{-\int_{\tau}^{t} \alpha h(s) \, ds} \, d\tau + \int_{t-T_B}^{t} \psi(\tau) e^{-\int_{\tau}^{t} \alpha h(s) \, ds} \, d\tau.
\]

(4.48)

Since \( \tau \leq T_B \), then when \( t > T_A + T_B \) then \( t > T_A + \tau \), therefore

\[
P_B(t) = \int_{0}^{T_B} \{2M(t - \tau - T_A) + \psi(t - \tau)\} e^{-\int_{\tau}^{t} h(s) \, ds} \, d\tau,
\]

(4.49)

\[
= \int_{t-T_A-T_B}^{t-T_A} 2M(\tau) e^{-\int_{\tau}^{t} \alpha h(s) \, ds} \, d\tau + \int_{T-B}^{t} \psi(\tau) e^{-\int_{\tau}^{t} \alpha h(s) \, ds} \, d\tau.
\]

### 4.4.3 Equations for \( P_C(t) \)

By definition,

\[P_C(t) = \int_{0}^{T_C} n_C(t, \tau) \, d\tau.\]

If \( t < T_A \) then \( t < T_C \), using this together with the initial condition given in Equation (4.3) implies \( t \geq \tau \), therefore the upper limit of the integral may now be changed to \( t \). Furthermore, if \( t < T_A \) then \( t < T_A + \tau \, \forall \tau \) so from Equation (4.27),

\[n_C(t, \tau) = \int_{0}^{t-\tau} h(t - \tau - \sigma) \psi(\sigma) e^{-\int_{\tau}^{t-\tau} h(s) \, ds} \, d\sigma.\]

Thus for \( t < T_A \)

\[P_C(t) = \int_{0}^{t} \int_{0}^{t-\tau} h(t - \tau - \sigma) \psi(\sigma) e^{-\int_{\tau}^{t-\tau} h(s) \, ds} \, d\sigma \, d\tau.\]

(4.50)

The order of integration may now be changed but as a result the limits will also change. The new limits may be found by considering a graphical representation of the region of integration as in Figure 4.2.
From Figure 4.2 it can be seen that
\[ \int_{0}^{t} \int_{0}^{t-\tau} d\sigma d\tau = \int_{0}^{t} \int_{0}^{t-\sigma} d\tau d\sigma, \]
therefore Equation (4.50) may be written as
\[ P_C(t) = \int_{0}^{t} \int_{0}^{t-\sigma} h(t - \tau - \sigma) \psi(\sigma) e^{-\int_{0}^{t-\tau-\sigma} h(s) ds} d\tau d\sigma. \] (4.51)

This may be simplified further to
\[ P_C(t) = \int_{0}^{t} \psi(\sigma) \int_{0}^{t-\sigma} h(t - \tau - \sigma) e^{-\int_{0}^{t-\tau-\sigma} h(s) ds} d\tau d\sigma. \] (4.52)

If \( T_A \leq t < T_C \) then as for the previous case the upper limit of the integral for \( P_C(t) \) may 
be changed to \( t \). Also, \( \exists t \) and \( \tau \) that satisfy \( t \geq T_A + \tau \) and \( t < T_A + \tau \) therefore the 
integral for \( P_C(t) \) for this range of \( t \) may be divided into two parts
\[ P_C(t) = \left\{ \begin{array}{ll}
\int_{0}^{t} \int_{0}^{t-\tau} n_C(t, \tau) d\tau d\sigma & \text{part (i)} \\int_{t-T_A}^{t} n_C(t, \tau) d\tau & \text{part (ii)}
\end{array} \right\} \] (4.53)

For part (i) of Equation (4.53) \( T_A + \tau \leq t \). Since \( t < T_C \) it is clear \( t < T_B + \tau \), thus for 
part (i) \( t \) satisfies \( T_A + \tau \leq t < T_B + \tau \), therefore from Equation (4.27)
\[ n_C(t, \tau) = \int_{0}^{t-\tau-T_A} 2h(t - \tau - \sigma - T_A) M(\sigma) e^{-\int_{0}^{\tau-\sigma-T_A} h(s) ds} d\sigma \]
\[ + \int_{0}^{t-\tau} h(t - \tau - \sigma) \psi(\sigma) e^{-\int_{0}^{\tau-\sigma} h(s) ds} d\sigma. \]
For part (ii) of Equation (4.53) \( t < T_A + \tau \). Also \( T_A \leq t \) thus from Equation (4.27)

\[
n_C(t, \tau) = \int_{t-\tau}^{t} h(t - \tau - \sigma) \psi(\sigma) e^{-\int_{0}^{\sigma} h(s) ds} d\sigma.
\]

Therefore, if \( T_A \leq t < T_C \) then

\[
P_C(t) = \int_{0}^{t-T_A} \int_{0}^{t-\tau-T_A} 2h(t - \tau - \sigma - T_A) M(\sigma) e^{-\int_{0}^{\tau} h(s) ds} d\sigma d\tau
\]

\[
+ \int_{0}^{t-T_A} \int_{0}^{t-\tau} h(t - \tau - \sigma) \psi(\sigma) e^{-\int_{0}^{\sigma} h(s) ds} d\sigma d\tau
\]

\[
+ \int_{t-T_A}^{t} \int_{0}^{t-\tau} h(t - \tau - \sigma) \psi(\sigma) e^{-\int_{0}^{\sigma} h(s) ds} d\sigma d\tau,
\]

\[
= \int_{0}^{t-T_A} \int_{0}^{t-\tau-T_A} 2h(t - \tau - \sigma - T_A) M(\sigma) e^{-\int_{0}^{\tau} h(s) ds} d\sigma d\tau
\]

\[
+ \int_{0}^{t} \int_{0}^{t-\tau} h(t - \tau - \sigma) \psi(\sigma) e^{-\int_{0}^{\sigma} h(s) ds} d\sigma d\tau,
\]

\[(4.54)\]

\[
= \int_{0}^{t-T_A} \int_{0}^{t-\tau-T_A} 2h(t - \tau - \sigma - T_A) M(\sigma) e^{-\int_{0}^{\tau} h(s) ds} d\tau d\sigma
\]

\[
+ \int_{0}^{t} \int_{0}^{t-\sigma} h(t - \tau - \sigma) \psi(\sigma) e^{-\int_{0}^{\sigma} h(s) ds} d\tau d\sigma,
\]

\[
= \int_{0}^{t-T_A} M(\sigma) \int_{0}^{t-\tau-T_A} 2h(t - \tau - \sigma - T_A) e^{-\int_{0}^{\tau} h(s) ds} d\tau d\sigma
\]

\[
+ \int_{0}^{t} \psi(\sigma) \int_{0}^{t-\sigma} h(t - \tau - \sigma) e^{-\int_{0}^{\sigma} h(s) ds} d\tau d\sigma.
\]
For $T_C < t < T_B$

$$P_C(t) = \int_0^{t-T_A} \int_0^{t-\tau-T_A} 2h(t - \tau - \sigma - T_A)M(\sigma)e^{-\int_{\tau}^{\sigma} h(s) ds} d\sigma d\tau$$

$$+ \int_0^{t-T_A} \int_0^{t-\tau} h(t - \tau - \sigma)\psi(\sigma)e^{-\int_{\tau}^{\sigma} h(s) ds} d\sigma d\tau$$

$$+ \int_{t-T_A}^{T_C} \int_0^{t-\tau} h(t - \tau - \sigma)\psi(\sigma)e^{-\int_{\tau}^{\sigma} h(s) ds} d\sigma d\tau,$$

$$= \int_0^{t-T_A} \int_0^{t-\tau-T_A} 2h(t - \tau - \sigma - T_A)M(\sigma)e^{-\int_{\tau}^{\sigma} h(s) ds} d\sigma d\tau$$

$$+ \int_0^{t-T_C} \int_0^{T_C} h(t - \tau - \sigma)\psi(\sigma)e^{-\int_{\tau}^{\sigma} h(s) ds} d\sigma d\tau$$

$$+ \int_{t-T_C}^{t-\tau} \int_0^{t-\tau} h(t - \tau - \sigma)\psi(\sigma)e^{-\int_{\tau}^{\sigma} h(s) ds} d\sigma d\tau,$$

$$= \int_0^{t-T_A} M(\sigma) \int_0^{t-\tau-T_A} 2h(t - \tau - \sigma - T_A)e^{-\int_{\tau}^{\sigma} h(s) ds} d\sigma d\tau$$

$$+ \int_0^{t-T_C} \psi(\sigma) \int_0^{T_C} h(t - \tau - \sigma)e^{-\int_{\tau}^{\sigma} h(s) ds} d\sigma d\tau$$

$$+ \int_{t-T_C}^{t-\tau} \psi(\sigma) \int_0^{t-\tau} h(t - \tau - \sigma)e^{-\int_{\tau}^{\sigma} h(s) ds} d\sigma d\tau.$$
Details of the change of variables are given in Appendix D.
For \( t \geq T_B \) it is necessary to consider the ranges \( T_B \leq t < T_A + T_C, T_A + T_C \leq t < T_A + T_B, T_A + T_B \leq t < T_B + T_C, T_B + T_C \leq t < T_A + T_B + T_C, t \geq T_A + T_B + T_C \). After similar manipulations of the expression for \( P_C(t) \) the following are obtained.

For \( T_B \leq t < T_A + T_C \)

\[
P_C(t) = \int_0^{t-T_A} M(\sigma) \int_0^{t-T_A} 2h(t - \tau - \sigma - T_A)e^{-\int_0^{t-\tau-\sigma-T_A} h(s)ds} d\tau d\sigma \\
+ \int_0^{t-T_C} \psi(\sigma) \int_0^{T_C} h(t - \tau - \sigma) e^{-\int_0^{t-\tau-\sigma} h(s)ds} d\tau d\sigma \\
+ \int_{t-T_C}^{t} \psi(\sigma) \int_0^{t-\sigma} h(t - \tau - \sigma) e^{-\int_0^{t-\tau-\sigma} h(s)ds} d\tau d\sigma \\
- \int_0^{t-T_B} \psi(\sigma) \int_0^{t-\sigma-T_B} h(t - \tau - \sigma) e^{-\int_0^{t-\tau-\sigma-T_B} h(s)ds} d\tau d\sigma.
\]

(4.56)

For \( T_A + T_C \leq t < T_A + T_B \)

\[
P_C(t) = \int_0^{t-T_C-T_A} M(\sigma) \int_0^{T_C} 2h(t - \tau - \sigma - T_A)e^{-\int_0^{t-\tau-\sigma-T_A} h(s)ds} d\tau d\sigma \\
+ \int_{t-T_C-T_A}^{t-T_A} M(\sigma) \int_0^{t-\sigma-T_A} 2h(t - \tau - \sigma - T_A)e^{-\int_0^{t-\tau-\sigma-T_A} h(s)ds} d\tau d\sigma \\
+ \int_0^{t-T_C} \psi(\sigma) \int_0^{T_C} h(t - \tau - \sigma) e^{-\int_0^{t-\tau-\sigma} h(s)ds} d\tau d\sigma \\
+ \int_{t-T_C}^{t} \psi(\sigma) \int_0^{t-\sigma} h(t - \tau - \sigma) e^{-\int_0^{t-\tau-\sigma} h(s)ds} d\tau d\sigma \\
- \int_0^{t-T_B} \psi(\sigma) \int_0^{t-\sigma-T_B} h(t - \tau - \sigma) e^{-\int_0^{t-\tau-\sigma-T_B} h(s)ds} d\tau d\sigma.
\]

(4.57)
For $T_A + T_B \leq t < T_B + T_C$

\[
P_C(t) = \int_0^{t-T_A-T_C} M(\sigma) \int_0^{T_C} 2h(t - \tau - \sigma - T_A)e^{-\int_0^{\tau+\sigma-T_A} h(s) \, ds} \, d\tau \, d\sigma
\]

\[
+ \int_{t-T_A-T_C}^{t-T_A} M(\sigma) \int_0^{t-T_A-\sigma} 2h(t - \tau - \sigma - T_A)e^{-\int_0^{\tau+\sigma-T_A} h(s) \, ds} \, d\tau \, d\sigma
\]

\[
- \int_0^{t-T_A-T_B} M(\sigma) \int_0^{t-T_A-T_B-\sigma} 2h(t - \tau - \sigma - T_A)e^{-\int_0^{\tau+\sigma-T_A} h(s) \, ds} \, d\tau \, d\sigma
\]

\[
+ \int_0^{t-T_C} \psi(\sigma) \int_0^{T_C} h(t - \tau - \sigma)e^{-\int_0^{\tau+\sigma} h(s) \, ds} \, d\tau \, d\sigma
\]

\[
+ \int_{t-T_C}^{t} \psi(\sigma) \int_0^{t-\sigma} h(t - \tau - \sigma)e^{-\int_0^{\tau+\sigma} h(s) \, ds} \, d\tau \, d\sigma
\]

\[
- \int_{t-T_C}^{t} \psi(\sigma) \int_0^{t-T_B-\sigma} h(t - \tau - \sigma)e^{-\int_0^{\tau+\sigma} h(s) \, ds} \, d\tau \, d\sigma.
\]

For $T_B + T_C \leq t < T_A + T_B + T_C$

\[
P_C(t) = \int_0^{t-T_A-T_C} M(\sigma) \int_0^{T_C} 2h(t - \tau - \sigma - T_A)e^{-\int_0^{\tau+\sigma-T_A} h(s) \, ds} \, d\tau \, d\sigma
\]

\[
+ \int_{t-T_A-T_C}^{t-T_A} M(\sigma) \int_0^{t-T_A-\sigma} 2h(t - \tau - \sigma - T_A)e^{-\int_0^{\tau+\sigma-T_A} h(s) \, ds} \, d\tau \, d\sigma
\]

\[
- \int_0^{t-T_A-T_B} M(\sigma) \int_0^{t-T_A-T_B-\sigma} 2h(t - \tau - \sigma - T_A)e^{-\int_0^{\tau+\sigma-T_A} h(s) \, ds} \, d\tau \, d\sigma
\]

\[
+ \int_0^{t-T_C} \psi(\sigma) \int_0^{T_C} h(t - \tau - \sigma)e^{-\int_0^{\tau+\sigma} h(s) \, ds} \, d\tau \, d\sigma
\]

\[
+ \int_{t-T_C}^{t} \psi(\sigma) \int_0^{t-\sigma} h(t - \tau - \sigma)e^{-\int_0^{\tau+\sigma} h(s) \, ds} \, d\tau \, d\sigma
\]

\[
- \int_{t-T_C}^{t} \psi(\sigma) \int_0^{t-T_B-\sigma} h(t - \tau - \sigma)e^{-\int_0^{\tau+\sigma} h(s) \, ds} \, d\tau \, d\sigma.
\]
For \( t \geq T_A + T_B + T_C \)

\[
P_C(t) = \int_{t-T_A-T_B-T_C}^{t-T_A-T_C} M(\sigma) \int_{0}^{T_C} 2h(t-\tau-\sigma-T_A)e^{-\int_{0}^{\tau} h(s)ds} d\tau d\sigma \\
+ \int_{t-T_A-T_C}^{t-T_A} M(\sigma) \int_{0}^{t-T_A-\sigma} 2h(t-\tau-\sigma-T_A)e^{-\int_{0}^{\tau} h(s)ds} d\tau d\sigma \\
- \int_{t-T_A-T_B-T_C}^{t-T_A-T_B} M(\sigma) \int_{0}^{t-T_A-T_B-\sigma} 2h(t-\tau-\sigma-T_A)e^{-\int_{0}^{\tau} h(s)ds} d\tau d\sigma \\
+ \int_{t-T_B-T_C}^{t-T_C} \psi(\sigma) \int_{0}^{T_C} h(t-\tau-\sigma)e^{-\int_{0}^{\tau} h(s)ds} d\tau d\sigma \\
+ \int_{t-T_C}^{t-\sigma} \psi(\sigma) \int_{0}^{t-\sigma} h(t-\tau-\sigma)e^{-\int_{0}^{\tau} h(s)ds} d\tau d\sigma \\
- \int_{t-T_B-T_C}^{t-T_B-\sigma} \psi(\sigma) \int_{0}^{t-T_B-\sigma} h(t-\tau-\sigma)e^{-\int_{0}^{\tau} h(s)ds} d\tau d\sigma.
\]

(4.60)

### 4.4.4 Equations for \( P_D(t) \)

By definition,

\[
P_D(t) = \int_{0}^{t} n_D(t, \tau) d\tau.
\]

If \( t \leq T_B \) then no cells have yet had chance to enter the \( D \) compartment, therefore \( P_D(t) = 0 \). For \( t \geq T_B \) then there are two ranges which need to be considered, namely \( T_B \leq t < T_A + T_B \) and \( t \geq T_A + T_B \). Proceeding in a similar manner as for \( P_C(t) \) leads to the following expressions for \( P_D(t) \). For \( T_B \leq t < T_A + T_B \)

\[
P_D(t) = e^{-\int_{0}^{T_B} h(s)ds} \int_{0}^{t-T_B} \psi(\tau) d\tau.
\]

(4.61)

For \( t \geq T_A + T_B \)

\[
P_D(t) = e^{-\int_{0}^{T_B} h(s)ds} \int_{0}^{t-T_A-T_B} 2M(\tau) d\tau \\
+ e^{-\int_{0}^{T_B} h(s)ds} \int_{0}^{t-T_B} \psi(\tau) d\tau.
\]

(4.62)
4.5 Integral Equations Summary

As discussed at the start of this chapter no analysis of these integral equations has been undertaken and they are included here to illustrate the availability of different techniques for modelling a population of cells using a population balance framework. The key equation in this integral equation approach is given by Equation (4.38). In order to solve Equation (4.38), it is necessary to solve the equations for $M(t)$ for earlier $t$, these are given by Equations (4.32-4.36). Once Equation (4.38) is solved the cell densities may readily be calculated. Numerical calculations involving the integral equations may provide a faster alternative to using a finite difference scheme for solving the PDEs directly but this has not been investigated further.
Chapter 5

Pharmacokinetic and Pharmacodynamic Models

Pharmacokinetics (PK) and pharmacodynamics (PD) are the branches of pharmacology which are concerned with how a drug interacts with a biological environment. Pharmacokinetics is the study of the effects of a biological system on a drug including the absorption of the drug, the distribution of the drug through the biological system and the excretion of the drug from the system. Pharmacodynamics is the study of the effects of a drug on a biological system.

Pharmacokinetic/pharmacodynamic (PKPD) modelling is an attempt to model the interaction of a drug with a particular biological environment. Using a PKPD model it is often possible to describe the interactions between the drug and the biological system in a simple, concise way. Once an appropriate model has been found it may be used to explain how the drug is interacting with the biological system. A major advantage of using PKPD models over actual experimental data is that a wider range of parameter values and scenarios may be considered and the models are not subject to the same level of uncertainty and error which can never be fully eliminated from experiments.

This chapter starts by giving a brief overview of how PKPD may be modelled. In the case of pharmacodynamics the way in which this may be incorporated directly into a cell cycle model is discussed. A brief introduction to widely used pharmacokinetic modelling is then undertaken. Firstly, a single compartment model for a single intravenous bolus dose is discussed, together with the resulting analytic solution. A single compartment
model for a single period of constant rate intravenous infusion is then considered. For multiple periodic doses an iterative map of ODEs and initial conditions is discussed, but not solved. For drugs administered extravascularly there is a delay before the drug enters the blood plasma. This delay means an extra compartment is necessary to accurately model the drug distribution. A simple two compartment model for a single extravascular dose is discussed, together with the resulting analytic solution for the concentration of the drug in the plasma. It is explained how the effects of bioavailability may be included and what effect this would have on the resulting solution. The chapter concludes with a brief discussion of the type of PKPD model which will be used in conjunction with a cell cycle model in Chapter 6.

5.1 Mathematical Modelling of Pharmacokinetics and Pharmacodynamics

PKPD models vary considerably in their complexity and level of detail, however it is often possible to gain qualitative insight using a model which still retains the essential properties of the interactions between a drug and it’s environment.

The PKPD modelling for a particular drug needs may be divided into two parts. The first of these relates to the pharmacokinetics.

The first consideration is how the drug is administered into the system. If the drug is administered intravenously it enters the blood plasma and hence the system directly. This means the model does not need to take into account the absorption of the drug by the body. Furthermore, if the drug is administered as a single bolus intravenous dose then the infusion period may be considered to be negligible in comparison to the other time scales in the model thus, the administration of the drug may be included as initial conditions in the model, this type of model is discussed further in Section 5.1.1. If the drug is infused over a period of time then this is not the case and the model needs to be broken into two phases, during infusion and post infusion, this is also considered in Section 5.1.1. Drugs administered orally, or by other means, need the absorption of the drug into the plasma to be included within the model, this scenario is examined in Section 5.1.2.

Once the drug is in the system how the drug leaves needs consideration. The clearance of the drug consists of several processes however, for the purposes of most models these
may normally be grouped together into one term. Unless the concentration is above 
the clearance capacity of the system the rate of clearance may be assumed to be first 
order. For higher concentrations a Michaelis-Menten term may be used to model the 
clearance [26].

Whilst in the system the distribution of the drug and its interaction with the system may 
also be included in the model.

Simple pharmacokinetic models with and without absorption are shown in Figures 5.1 
and 5.2.

![Figure 5.1: Pharmacokinetic Model Without Absorption.](image)

![Figure 5.2: Pharmacokinetic Model With Absorption.](image)

The pharmacodynamic part of the modelling is included in the model for the main bio-
logical system. How it is included depends on the actions of the drug and also the system 
on which it is acting.

As the biological system being considered is a population of cancerous cells the purpose 
of the drug is to reduce the rate of growth of the population. There are two ways the 
pharmacodynamics may be included.
If a drug is cytotoxic then it is toxic to the cells. This results in some of the cells dying directly as a result of the drug. Some drugs do not kill cells directly, instead they inhibit a cell’s ability to divide and successfully undergo mitosis, this is known as a cytostatic effect. The proportion of cells that are affected by a drug is dependent on factors such as dosage and the drug’s efficacy.

Cytotoxic drugs may be modelled by introducing a function linking the drug concentration to its efficacy. This may be added to the equations for the cell population. The resulting term is likely to be negative as it represents a decline in the number of cells.

If the drug being used is cytostatic then instead of a simple doubling at cell division a function may be used to govern the average number of daughter cells each cell within the population will produce when exposed to a particular concentration of the drug. Clearly, a cell either divides into two daughter cells or it does not and such averaging approaches are only suitable when the population of cells being considered is large enough so each individual division is not important and an average value of divisions is meaningful.

5.1.1 Simple Intravenous Models

If the drug is administered intravenously it immediately enters the blood plasma and hence the biological system. This means models for intravenous delivery do not need to take into account the absorption of the drug by the body. A graphical representation of this is shown in Figure 5.1. Furthermore, if the drug is administered as a single bolus intravenous dose then the infusion period may often be considered to be negligible in comparison to the other time scales in the model such as the rate at which the drug leave the system. Thus, the administration of the drug may be included as initial conditions in the model. If the drug is infused over a period of time then this is not the case and the model may be broken into two phases, during infusion and post infusion.

Bolus Dose Model

The most simple type of pharmacokinetic model considers a single, bolus dose which leaves the system at a known rate. This results in a first order ordinary differential equation for the concentration of the drug in the system. Let $D(t)$ denote the drug concentration at time $t$ in a compartment representing the biological system with volume $V$. If the drug
is known to leave the system in a linear way, relative to its concentration, and an initial bolus dose of \( D_I \) is given at \( t = 0 \) then the concentration of the drug may be expressed as
\[
\frac{d D(t)}{dt} = -K D(t) \quad \text{and} \quad D(0) = \frac{D_I}{V}, \tag{5.1}
\]
where \( K \) is a rate constant associated with how fast the drug is removed from the system either by leaving the system or being irreversibly changed via interactions with the system, such as cell absorption or binding. Equation (5.1) may easily be solved to give
\[
D(t) = \frac{D_I}{V} e^{-Kt}, \tag{5.2}
\]
for the drug concentration, \( D(t) \), at any given time.

**Intravenous Infusion Model**

Delivering the drug via a constant rate infusion is preferable to a single bolus dose or extravascular methods, especially when the drug has a narrow therapeutic index\(^1\) as a constant rate infusion allows greater control over the input of the drug. Regular monitoring allows an accurate picture of the concentration in the biological system to be obtained. If the drug is infused, at a constant rate \( R \), which is greater than the rate at which the drug leaves the system \( K \), then the concentration of the drug in the system will increase over the period of infusion. Once the infusion has stopped the bolus dose model, previously described, may be used with \( D_I \) equal to the concentration of the drug at the end of the infusion period.

Let \( D(t) \) denote the drug concentration at time \( t \) in a compartment representing the biological system with volume \( V \). If the drug is known to leave the system in a linear way, relative to its concentration at rate \( K \), and for \( t \in [0, T_I) \) the drug is infused at a constant rate \( R \) then if the initial drug concentration in the system is assumed to be zero the concentration of the drug may be expressed as
\[
\frac{d D(t)}{dt} = \begin{cases} \frac{R}{V} - K D(t) & \text{for } 0 \leq t < T_I, \\ -K D(t) & \text{for } t \geq T_I, \end{cases} \quad \text{and} \quad D(0) = 0. \tag{5.3}
\]

\(^1\)The therapeutic index is a measure of the range of concentration for which the drug is therapeutically effective but not toxic. For humans the therapeutic index is given as the ratio of the dose which produces a toxicity response in 50% of the population and the dose which produces a therapeutic effect in 50% of the population.
During the period of constant infusion, \( t \in [0, T_I) \), the first part of Equation (5.3) and the initial condition apply. This ODE has the analytic solution

\[
D(t) = \frac{R}{K_V} \left( 1 - e^{-K t} \right) \quad \text{for} \quad 0 \leq t < T_I. \tag{5.4}
\]

After the infusion has finished then the second part of (5.3) applies, with the initial condition being given by setting \( t = T_I \) in Equation (5.4), i.e. \( D(T_I) = \frac{R}{K_V} (1 - e^{-K T_I}) \). Solving for \( t \in [T_I, \infty) \) gives

\[
D(t) = \frac{R}{K_V} \left( e^{K T_I} - 1 \right) e^{-K t} \quad \text{for} \quad T_I \leq t. \tag{5.5}
\]

A sketch of a typical drug concentration administered via constant infusion is shown in Figure 5.3. If the same equal dose is given via constant infusion and a single bolus dose it is possible to derive a relationship for the back extrapolated intercept for the concentration of the constant infusion and the single bolus dose [26] and [27]. Let \( C_I \) denote the back extrapolated intercept of the constant infusion concentration curve, post infusion. Let \( C_B \) denote the concentration immediately after the bolus dose (If the dose is \( D_I \) clearly \( C_B = \frac{D_I}{V} \)). By setting \( t = 0 \) in Equation (5.5) gives

\[
C_I = \frac{R}{K_V} \left( e^{K T_I} - 1 \right), \tag{5.6}
\]

but the total dose given is the rate \( R \) multiplied by the length of time of the infusion period \( T_I \), and this is equal to the single bolus dose \( D_I \). Therefore \( R = \frac{D_I}{T_I} = \frac{C_B V}{T_I} \), thus Equation (5.6) may be rewritten as

\[
C_I = \frac{C_B}{K T_I} \left( e^{K T_I} - 1 \right), \tag{5.7}
\]

or

\[
C_B = \frac{C_I K T_I}{e^{K T_I} - 1}. \tag{5.8}
\]

This result is shown in [26] and [27] and applies to all mammillary compartment models [27].

5.1.2 Simple Absorption Models

For drugs administered by any other means other than intravenously there will be some delay before the drug distributes through the system and into the plasma and tissues.
Figure 5.3: A Sketch of a Typical Plasma Drug Concentration Curve When the Dose is Administered Via Constant Infusion.
Because of this delay a single compartment model is often not sufficient to predict the concentration. By introducing more compartments additional exponential terms are introduced into the prediction for the concentration. These additional terms represent different rates for the drug distribution. This often provides a better fit to experimental data.

A two compartment model as depicted in Figure 5.2 may be used as a first approximation for an absorption model. For a single extravascular, bolus dose \( D_E \) this model is described by the following pair of coupled ODEs.

\[
\frac{dD_O(t)}{dt} = -K_O D_O(t) \quad \text{and} \quad D_O(0) = \frac{D_E}{V}, \quad (5.9a)
\]

\[
\frac{dD_P(t)}{dt} = K_O D_O(t) - K_P D_P(t) \quad \text{and} \quad D_P(0) = 0, \quad (5.9b)
\]

where \( D_O(t) \) and \( D_P(t) \) are the concentrations of the drug in the outer compartment and plasma respectively and \( K_O \) and \( K_P \) are the rates at which the drug leaves the outer compartment and plasma respectively. Equation (5.9a) is the same as Equation (5.1) and hence has the solution

\[
D_O(t) = \frac{D_E}{V} e^{-K_O t}. \quad (5.10)
\]

\( D_O(t) \) may now be substituted into Equation (5.9b) to give

\[
\frac{dD_P(t)}{dt} = K_O \frac{D_E}{V} e^{-K_O t} - K_P D_P(t) \quad \text{and} \quad D_P(0) = 0, \quad (5.11)
\]

as the equation describing the drug concentration in the plasma \( D_P(t) \). Equation (5.11) may be solved yielding

\[
D_P(t) = \frac{K_O D_E (e^{-K_P t} - e^{-K_O t})}{(K_O - K_P) V}. \quad (5.12)
\]

This result appears in [26]. Providing \( K_O \gg K_P \) this shows a two compartment model may be approximated by a single compartment as \( e^{-K_P t} \) is the dominant term. A sketch of a typical concentration curve in the plasma compartment is shown in Figure 5.4.

In reality not all the drug that is in the outer compartment enters the plasma as shown in Figure 5.5. For example, if the outer compartment represents the gut then a small amount of the drug will be lost via excretion and degradation.
Figure 5.4: A Sketch of a Typical Plasma Drug Concentration Curve When the Dose is Administered Via an Absorption Process.
The proportion of the drug which leaves the out compartment and enters the plasma is known as the bioavailability $B$. Mathematically this may expressed as

$$B = \frac{K_O}{K_O + K_{lost}},$$

(5.13)

where $K_{lost}$ is the rate constant representing drug which leaves via other means, such as degradation. Equation (5.9b) may be modified to take this into account simply by scaling the input term appropriately

$$\frac{dD_P(t)}{dt} = K_O BD_O(t) - K_P D_P(t) \quad \text{and} \quad D_P(0) = 0.\quad (5.14)$$

Other two compartment models such as that depicted in Figure 5.6 may provide a more accurate representation of the concentration of the drug if it is administered extravascularly. The corresponding solution of which is similar to that given Equation (5.12) in as much as it contains two exponential terms.
Figure 5.6: Pharmacokinetic Absorption Model With Loss From the Outer Compartment and Two Way Drug Diffusion.

It is possible to create more sophisticated and detailed models using more compartments. The rates at which the drug pass between the compartments and volumes of the compartments may also be time dependent.

### 5.1.3 Periodic doses

A drug which is administered periodically may have its concentration modelled using an iterative map. The first step in constructing the iterative map is to use a periodic function instead of the constant infusion $R$ as described in Section 5.1.1 If the infusion rate is now a function of time, $R(t)$, then Equation (5.3) may be modified to

$$
\frac{dD(t)}{dt} = \begin{cases} 
\frac{R(t)}{V} - KD(t) & \text{for } T_{I_{2n}} < t \leq T_{I_{2n+1}} \text{ for } 1 \leq n \leq N, \\
-KD(t) & \text{for } T_{I_{2n+1}} < t \leq T_{I_{2n+2}} \text{ for } 1 \leq n < N \text{ and } t \geq T_{I_{2n+1}},
\end{cases}
$$

and $D(0) = 0.$  \hspace{1cm} (5.15)

where $\{n \in \mathbb{N}^0 \mid n < N\}$ and $N > 1$ is the number of infusions. It follows $T_{I_{2n}}$ are the times the infusions start and $T_{I_{2n+1}}$ are the corresponding times the infusions finish. If $N$ is small then it is possible to construct a piecewise continuous solution directly from Equation (5.15), similar to the solution given by Equations (5.4) and (5.5). For large $N$ an iterative map is used for which an analytic solution may be difficult to obtain.

During periods of infusion if the rate at which the drug is administered is at a constant rate $R$ then the the drug concentration, $D_n(t)$, during the $n^{th}$ period of infusion/post infusion out of a total $N$ infusions is given by

$$
\frac{dD_n(t)}{dt} = \begin{cases} 
\frac{R}{V} - KD_n(t) & \text{for } 0 < t \leq T_{I_n}, \\
-KD_n(t) & \text{for } T_{I_n} < t \leq T_n,
\end{cases} \hspace{1cm} \text{and } D_n(0) = D_{n-1}(T_n), \hspace{1cm} (5.16)
$$

where $T_{I_n}$ is the length of the infusion period in the $n^{th}$ infusion and $T_n$ is the time that the $(n+1)^{st}$ infusion starts. Clearly for $t \in (T_{I_n}, T_n]$ there is no infusion. It should be noted that at the start of each new mapping $t$ is reset to zero. For completeness the initial
condition $D_1(0) = 0$ together with the equation

$$\frac{d D_{END}(t)}{dt} = -K D_{END}(t) \quad \text{and} \quad D_{END}(0) = D_N(T_N), \quad (5.17)$$

for $t \geq \sum_{T} T_N$ are required.

### 5.2 Pharmacokinetic and Pharmacodynamic Model Summary

There are a wide variety of compartment based models available to accurately represent drug concentration for drugs delivered by both intravenous and extravenuous methods. As a first approximation to gain a qualitative understanding of how a system behaves it is possible to use a single compartment model. The addition of further compartments makes the analysis of the drug concentration more difficult and since the additional compartments add exponential terms as long as $K_O \gg K_P$ then a single exponential term is a good first approximation of the multi-compartment model. It was shown in Section 5.1.1 that it was possible to back extrapolate from the graph of the concentration caused by a constant rate infusion to obtain the equivalent bolus dose which would give the concentration curve post infusion. As such to simplify the model further the dose may be assumed to be administered in a single bolus dose.
Chapter 6

A Cell Cycle Model with Drug Interaction

In Section 3.1 a four compartment, ODE cell cycle model was constructed. By comparing the phase distributions given by PDE and ODE models it was shown in Section 3.6 that an ODE model was sufficient to gain qualitative information concerning how the population of cells grow. This chapter starts with Section 6.1 extending this four compartment, ODE cell cycle model to account for the ways in which drugs may interact with the population of cells. In Chapter 5 a number of basic PKPD models were considered. It was concluded in Section 5.2 that to gain a first approximation for most PKPD situations a single bolus, intravenous dose model would suffice. The purpose of this model is to develop a method for obtaining a semi-analytical solution which may be used to gain a qualitative understanding of the way in which different types of drug-cell interactions effect a given population. Since only a qualitative understanding of the system is sought it is therefore possible to combine the ODE model described in Section 3.1 with a simple PKPD model similar to that described in Section 5.1.1.

As previously discussed in Section 5.1 drugs may effect a cell in a number of different ways. It is therefore sensible to consider two separate models, one for each of the pharmacodynamic processes by which chemotherapy drugs effect a population of cells, namely cytostasis and cytotoxicity. In Section 6.2 the model is modified to take into account the effects of a cytotoxic drug on the population of cells. The modified system of equations are presented and non-dimensionalised. It is observed that the model may be divided into
three categories dependent on the order of one of the parameters of the system. For one of these cases an asymptotic approach cannot be used. This case corresponds to a region of parameter space which is unlikely to be of biological interest and is not considered further, leaving only two cases to be considered. It is shown one of these has a trivial solution, leaving only one of interest. For this remaining case (Case I) it is noted that for the special case detailed in Section 6.1 an analytic solution may be found. However, for the generic model it may not be possible to find an analytic solution. As such, a technique for obtaining an approximate solution is given. This technique is first demonstrated on a simpler system, of two ODEs, which has similarities to the biological system of interest. This enables the approximation to easily be compared with the analytical solution. Once the technique has been verified on the simple system it is used on the cytotoxic model and the results compared once more to the analytical solution.

Section 6.3 follows a similar format to Section 6.2 with the model now modified to take into account the effects of a cytostatic drug on the population of cells. As this scenario is worked through a number of similarities between the cytotoxic and cytostatic model are noted.

In Section 6.4 the cytotoxic and cytostatic models are compared. It is shown that for long term qualitative predictions both models produce the same results. The rationale for why this is the case is then discussed.

The chapter concludes with a summary of the findings.

6.1 Outline of Model

The model described in this section combines the four compartment ODE model described in Section 2.1.2 with a simple PKPD model, similar to the single compartment intravenous model described in Section 5.1.1.

Consider a closed population of cells where no quiescent cells are present. As discussed in Section 2.1.2 such a population may be modelled as four compartments each of which contain one phase. The rate at which cells pass between each phase is given by $k_i, i \in \{G_1, S, G_2, M\}$. In Section 2.1.2 only the case where $k_i, \forall i$ are constants was considered. It is possible that this may not be the case, for example the rates at which cells progress between the compartments may be a function of nutrient levels or the concentration of
some drug. Furthermore, let the average number of new cells produced per cell division be \( P \), which is a function of the drug concentration \( D(t) \). The cell population density in each of the four compartments is governed by the equations

\[
\begin{align*}
\frac{dN_{G1}(t)}{dt} &= P(D(t))k_1(D(t))N_M(t) - k_2(D(t))N_{G1}(t) - f_1(D(t))N_{G1}(t), \\
\frac{dN_S(t)}{dt} &= k_2(D(t))N_{G1}(t) - k_3(D(t))N_S(t) - f_2(D(t))N_S(t), \\
\frac{dN_{G2}(t)}{dt} &= k_3(D(t))N_S(t) - k_4(D(t))N_{G2}(t) - f_3(D(t))N_{G2}(t), \\
\frac{dN_M(t)}{dt} &= k_4(D(t))N_{G2}(t) - k_1(D(t))N_M(t) - f_4(D(t))N_M(t),
\end{align*}
\]

(6.1)

where \( N_{G1}(t), N_S(t), N_{G2}(t) \) and \( N_M(t) \) are the number of cells in the four compartments at time \( t \) and \( D(t) \) is the drug concentration in the inter cellular media at time \( t \). The functions \( f_i(D(t)), i \in \{1, 2, 3, 4\} \) represent the cytotoxic effect of the drug on the population of cells. Due to the processes which occur internally in a cell during its progression through the cell cycle there may be certain parts of the cycle for which a cell is particularly vulnerable to the cytotoxic effects of a drug. Conversely, there may also be periods for which the cell’s immunity is increased, as such the functions, \( f_i(D(t)) \), may vary significantly. The drug concentration in the inter cellular media, \( D(t) \), may be modelled in a similar way to the single compartment, bolus dose PKPD model described in Section 5.1.1. Let the rate at which the drug enters the media be given by \( d_{in}(t) \). Also, let the rate at which the drug leaves the media via clearance be directly proportional to the amount of drug in the media. Let this clearance occur at the rate \( d_{out}(t) \). The amount of drug absorbed by the cells is given by a function of the respective cell densities and the amount of drug available \( g(N_{G1}(t), N_S(t), N_{G2}(t), N_M(t), D(t)) \). The drug concentration in the inter cellular media is now governed by the equation

\[
\frac{dD(t)}{dt} = d_{in}(t) - d_{out}(t)D(t) - g(N_{G1}(t), N_S(t), N_{G2}(t), N_M(t), D(t)).
\]

(6.2)

Equations (6.1) and (6.2), together with suitable boundary conditions, fully define the cell cycle population and the inter cellular drug concentration, i.e. the complete system under consideration. A graphical representation of this system is shown in Figure 6.1.
Figure 6.1: Four Compartment ODE Cell Cycle Model Incorporating Drug Interaction.

The purpose of this model is to develop a method for obtaining a semi-analytical solution
which may be used to gain a qualitative understanding of the way in which different types of drug-cell interactions effect a given population. Since only a qualitative understanding of the system is sought it is possible to make some assumptions which in turn simplify the system of equations. The assumptions made in this model are

- The drug is delivered in a single bolus dose.
- The rate of clearance is directly proportional to the amount of drug in the system.
- The drug is taken up equally by all cells, regardless of phase.
- The drug concentration is below the cell’s saturation level.
- Cells pass between each of the four compartments at a constant rate, which is independent of any external factors.

By assuming the drug is delivered in a single bolus dose, $d_{in}(t)$ may be incorporated into the initial conditions. This assumption, together with the clearance being directly proportional to the amount of drug in the system simplify Equation (6.2) to

$$\frac{dD(t)}{dt} = -d_{out}D(t) - g(N_{G1}(t), N_S(t), N_{G2}(t), N_M(t), D(t)), \quad (6.3)$$

where $d_{out}$ is now a constant and not a function of time. Since the drug is assumed to be absorbed equally by all cells and the drug concentration is below the level required for saturation the rate the drug is used up is first order with respect to the drug concentration, thus $g(N_{G1}(t), N_S(t), N_{G2}(t), N_M(t), D(t)) = \delta N_T(t)D(t)$ where $N_T(t)$ is the total cell population density at time $t$, and $\delta$ is the amount of the drug a cell absorbs per unit time. Equation (6.3) now becomes

$$\frac{dD(t)}{dt} = -d_{out}D(t) - \delta N_T(t)D(t). \quad (6.4)$$

By assuming that cells pass between each of the four compartments at a constant rate which is independent of any external factors, the constants $k_i(D(t))$ may be expressed as
\[ k_1(D(t)) = k_2(D(t)) = k_3(D(t)) = k_4(D(t)) = k. \] Equations (6.1) now become

\[
\frac{dN_{G1}(t)}{dt} = P(D(t))kN_M(t) - kN_{G1}(t) - f_1(D(t))N_{G1}(t),
\]

\[
\frac{dN_S(t)}{dt} = kN_{G1}(t) - kN_S(t) - f_2(D(t))N_S(t),
\]

\[
\frac{dN_{G2}(t)}{dt} = kN_S(t) - kN_{G2}(t) - f_3(D(t))N_{G2}(t),
\]

\[
\frac{dN_M(t)}{dt} = kN_{G2}(t) - kN_M(t) - f_4(D(t))N_M(t).
\] (6.5)

This last assumption may be relaxed to allow a more biologically realistic distribution of cells across the four compartments. In Appendix F the model detailed in Section 6.2 is worked through with this assumption removed.

Depending on how a drug interacts with the population it may be possible to make further assumptions, and thus simplify the model further.

### 6.1.1 Types of Drug Interaction

There are many ways in which a drug may interfere with the normal progression of a cell through the cell cycle. The effects of a drug may be broken down into two broad categories cytotoxic and cytostatic. Cytotoxic literally means toxic to cells and as such may be considered as directly killing cells and reducing the population. There are many ways in which the toxicity may actually kill a cell such as the breaking down of the cell membrane, inducing apoptosis\(^1\) or reducing the rate of growth of a cell to a point where it is no longer viable. However, the specific mechanism of cell death is not important for the purposes of this model. Cytostatic drugs inhibit the division of cells. Strictly speaking, cytostasis may also refer to inhibition of growth of cells but for the purposes of this analysis this type of effect is included under cytotoxicity. Many drugs have a synergistic effect consisting of both cytotoxic and cytostatic components, but for simplicity these effects are treated separately here.

---

\(^1\)Apoptosis is the genetically pre-programmed cell shut down process leading to the cell’s death.
6.2 The Effect of a Cytotoxic Drug Interaction on A Population of Cells

If a drug has a cytotoxic effect on a cell population then its effect may by phase dependent [2]. In extreme cases the drug is only effective for cells in one particular phase. The special case where the drug only effects cells in the $S$ phase is considered here; thus $f_1(D(t)) = f_3(D(t)) = f_4(D(t)) = 0$. Furthermore, let the drug effect cells in the $S$ phase in a way which is directly proportional to the drug concentration in the inter cellular media, hence $f_2(D(t)) = eD(t)$ where $e$ can be thought of as the efficacy of the drug. Since the drug only has a cytotoxic effect the average number of cells produced at each division is not dependent on the drug concentration and is therefore a constant, thus $P(D(t)) = 2$. Equations (6.5) now simplify to

$$\frac{dN_G1(t)}{dt} = 2kN_M(t) - kN_G1(t),$$

$$\frac{dN_S(t)}{dt} = kN_G1(t) - kN_S(t) - eD(t)N_S(t),$$

$$\frac{dN_G2(t)}{dt} = kN_S(t) - kN_G2(t),$$

$$\frac{dN_M(t)}{dt} = kN_G2(t) - kN_M(t).$$

Equations (6.4) and (6.6) together with suitable initial conditions now represent a simplified model with a cytotoxic drug interaction.

To complete the system initial conditions are required. It is assumed each of the four compartments contain the same number of cells; therefore

$$N_i(0) = \frac{N_T(0)}{4} \text{ where } i \in \{G_1, S, G_2, M\}.$$  \hspace{1cm} (6.7)

It should be noted that the initial conditions given in Equation (3.10) could have been chosen. However, since this model now considers the effects of a drug, which may have a large effect at the beginning of the model, such initial conditions may distort the effect of the drug due to the cells only being present in one of the four phases. Whilst assuming an equal distribution of cells is still unrealistic this does alleviate this issue. Let the initial drug concentration be given by

$$D(0) = D_I.$$ \hspace{1cm} (6.8)
6.2 The Effect of a Cytotoxic Drug Interaction on A Population of Cells

6.2.1 Non-Dimensionalisation of the Equations

As previously discussed in Section 3.2 any potential numerical problems caused by multi-scale parameters and variables may be removed by non-dimensionalising the system of equations. Furthermore, by non-dimensionalising the system of equations it may be possible to reduce the number of dependent parameters making the system more tractable. The first step in the non-dimensionalisation process is to introduce new, dimensionless variables $\tilde{t}, \tilde{D}(\tilde{t}), \tilde{N}_{G_1}(\tilde{t}), \tilde{N}_S(\tilde{t}), \tilde{N}_{G_2}(\tilde{t}), \tilde{N}_M(\tilde{t})$ and $\tilde{N}_T(\tilde{t})$ defined as

$$\tilde{t} = \frac{t}{b},$$  \hspace{1cm} (6.9a)

$$\tilde{N}_{G_1}(\tilde{t}) = \frac{N_{G_1}(t)}{a},$$  \hspace{1cm} (6.9b)

$$\tilde{N}_S(\tilde{t}) = \frac{N_S(t)}{a},$$  \hspace{1cm} (6.9c)

$$\tilde{N}_{G_2}(\tilde{t}) = \frac{N_{G_2}(t)}{a},$$  \hspace{1cm} (6.9d)

$$\tilde{N}_M(\tilde{t}) = \frac{N_M(t)}{a},$$  \hspace{1cm} (6.9e)

$$\tilde{N}_T(\tilde{t}) = \sum_i \tilde{N}_i(\tilde{t}),$$  \hspace{1cm} (6.9f)

$$\tilde{D}(\tilde{t}) = \frac{D(t)}{c}.$$  \hspace{1cm} (6.9g)

Where $a, b$ and $c$ are dimensional constants which may be arbitrarily chosen to simplify the system. Note, the cell densities in the different compartments are all scaled equally to avoid potentially misleading distributions. Substituting the non-dimensional variables into Equations (6.4) and (6.6) gives

$$\frac{a}{b} \frac{d\tilde{N}_{G_1}(\tilde{t})}{dt} = 2ak\tilde{N}_M(\tilde{t}) - ak\tilde{N}_{G_1}(\tilde{t}),$$

$$\frac{a}{b} \frac{d\tilde{N}_S(\tilde{t})}{dt} = ak\tilde{N}_{G_1}(\tilde{t}) - ak\tilde{N}_S(\tilde{t}) - ace\tilde{D}(\tilde{t})\tilde{N}_S(\tilde{t}),$$  \hspace{1cm} (6.10)

$$\frac{a}{b} \frac{d\tilde{N}_{G_2}(\tilde{t})}{dt} = ak\tilde{N}_S(\tilde{t}) - ak\tilde{N}_{G_2}(\tilde{t}),$$

$$\frac{a}{b} \frac{d\tilde{N}_M(\tilde{t})}{dt} = ak\tilde{N}_{G_2}(\tilde{t}) - ak\tilde{N}_M(\tilde{t}),$$
and
\[
\frac{c}{b} \frac{d\tilde{D}(t)}{dt} = -cd_{\text{out}} \tilde{D}(t) - \delta a c \tilde{N}_T(\tilde{t}) \tilde{D}(\tilde{t}). \tag{6.11}
\]

The corresponding initial conditions are now given by
\[
\tilde{N}_i(0) = \frac{\tilde{N}_T(0)}{4} \text{ where } i \in \{G_1, S, G_2, M\}, \tag{6.12}
\]
and
\[
\tilde{D}(0) = \tilde{D}_I \text{ where } \tilde{D}_I = \frac{D_I}{c}. \tag{6.13}
\]
Equations (6.10) and (6.11) may be re-arranged to give, upon dropping the tilde notation,
\[
\frac{dN_{G1}(t)}{dt} = 2bkN_M(t) - bkN_{G1}(t),
\]
\[
\frac{dN_S(t)}{dt} = bkN_{G1}(t) - bkN_S(t) - bceD(t)N_S(t),
\]
\[
\frac{dN_{G2}(t)}{dt} = bkN_S(t) - bkN_{G2}(t),
\]
\[
\frac{dN_M(t)}{dt} = bkN_{G2}(t) - bkN_M(t),
\]
and
\[
\frac{dD(t)}{dt} = -bd_{\text{out}}D(t) - \delta abN_T(t)D(t). \tag{6.15}
\]
The parameters \(b\) and \(c\) are now chosen as \(b = \frac{1}{k}\) and \(c = \frac{k}{c}\), thus, the system of equations now become
\[
\frac{dN_{G1}(t)}{dt} = 2N_M(t) - N_{G1}(t),
\]
\[
\frac{dN_S(t)}{dt} = N_{G1}(t) - N_S(t) - D(t)N_S(t),
\]
\[
\frac{dN_{G2}(t)}{dt} = N_S(t) - N_{G2}(t), \tag{6.16}
\]
\[
\frac{dN_M(t)}{dt} = N_{G2}(t) - N_M(t),
\]
and
\[
\frac{dD(t)}{dt} = -\frac{d_{\text{out}}}{k}D(t) - \frac{\delta a}{k}N_T(t)D_n(t). \tag{6.17}
\]
The parameter $a$ may be chosen arbitrarily, but a sensible choice is $a = \frac{N_T(0)}{4}$. This value is chosen such that the normalised initial conditions for the cell population densities in each of the four compartments are all one. It should be noted that $a \gg 1$. Since $d_{out}$ represents the clearance of the drug per unit time and $k$ represents the rate at which cells progress between the compartments $\frac{d_{out}}{k} = \mathcal{O}(1)$. The parameter $\delta$ represents the amount of drug absorbed per cell per unit time and as such may be assumed to be very small. It should be noted that since $\delta$ is dependent on the timescale used the $\delta$ used in the non-dimensional system has been scaled by a factor of $k$ compared to the original $\delta$ given in Equation (6.4). It is now helpful to introduce two new parameters $\lambda = \frac{d_{out}}{k}$ which is of order $\mathcal{O}(1)$ and $\epsilon = \frac{\delta}{k}$ whose order is unknown. Equation (6.17) may now be rewritten as

\[
\frac{dD(t)}{dt} = -\lambda D(t) - \epsilon N_T(t) D(t). \tag{6.18}
\]

The non-dimensionalised form of the initial conditions for the cell population densities are now

\[N_i(0) = 1 \text{ where } i \in \{G_1, S, G_2, M\}. \tag{6.19}\]

Thus Equations (6.16) and (6.18) together with the initial conditions given by Equations (6.13) and (6.19) fully define the non-dimensionalised system.

Since the order of $\epsilon$ is unknown there are three cases to consider

- **Case I : $\epsilon \ll 1$,**
- **Case II : $\mathcal{O}(\epsilon) = 1$,**
- **Case III : $\epsilon \gg 1$.**

### 6.2.2 Case I : $\epsilon \ll 1$

If $\epsilon \ll 1$, then since $N_T(0) = 4$ clearly $\epsilon N_T(t) \ll 1$ for some range of $t \geq 0$. Subsequently however $\epsilon N_T(t) = \mathcal{O}(1)$ or even $\epsilon N_T(t) \gg 1$ may occur. However, since $\lambda = \mathcal{O}(1)$ the growth rate of $N_T(t)$ is the same order as the decay rate of $D(t)$, so that when $N_T(t) \gg 1$ then $D(t) \ll 1$. Therefore, only $\epsilon N_T(t) \ll 1$ needs to be considered.

For the zeroth order approximation all terms with $\epsilon$ may be ignored. Thus Equation (6.18) decouples from the system and simplifies to

\[
\frac{dD(t)}{dt} = -\lambda D(t), \tag{6.20}
\]
6.2 The Effect of a Cytotoxic Drug Interaction on A Population of Cells

\[ D(t) = A e^{-\lambda t}, \]  
\[ (6.21) \]

where A is a constant. Using the initial condition given in Equation (6.13) gives \( D(t) = D_IE^{-\lambda t} \). This expression for \( D(t) \) may now be inserted back into Equations (6.16) giving

\[ \frac{dN_{G1}(t)}{dt} = 2N_M(t) - N_{G1}(t), \]  
\[ (6.22a) \]

\[ \frac{dN_S(t)}{dt} = N_{G1}(t) - N_S(t) - D_IE^{-\lambda t}N_S(t), \]  
\[ (6.22b) \]

\[ \frac{dN_{G2}(t)}{dt} = N_S(t) - N_{G2}(t), \]  
\[ (6.22c) \]

\[ \frac{dN_M(t)}{dt} = N_{G2}(t) - N_M(t). \]  
\[ (6.22d) \]

In order to analyse the solution of this system of equations it is helpful to convert them into a single fourth order equation. By differentiating Equation (6.22d) the second order equation

\[ \frac{d^2N_M(t)}{dt^2} = \frac{dN_{G2}(t)}{dt} - \frac{dN_M(t)}{dt}, \]  
\[ (6.23) \]

is obtained. Substituting the expression for the derivative of \( N_{G2}(t) \) from Equation (6.22c) gives

\[ \frac{d^2N_M(t)}{dt^2} = N_S(t) - N_{G2}(t) - \frac{dN_M(t)}{dt}. \]  
\[ (6.24) \]

It is now possible to eliminate \( N_{G2}(t) \) by making it the subject of Equation (6.22d) and substituting this expression into Equation (6.24) which leads to

\[ \frac{d^2N_M(t)}{dt^2} = -2\frac{dN_M(t)}{dt} - N_M(t) + N_S(t). \]  
\[ (6.25) \]

Proceeding in a similar manner it is possible to eliminate \( N_{G2}(t) \) and \( N_M(t) \) giving a single fourth order ODE for \( N_{G1} \), i.e.

\[ \frac{d^4N_{G1}(t)}{dt^4} + (4 + Di e^{-\lambda t}) \frac{d^3N_{G1}(t)}{dt^3} + (6 + 3Di e^{-\lambda t}) \frac{d^2N_{G1}(t)}{dt^2} \]

\[ + (4 + 3Di e^{-\lambda t}) \frac{dN_{G1}(t)}{dt} - (1 - Di e^{-\lambda t}) N_{G1}(t) = 0. \]  
\[ (6.26) \]
By utilising the \texttt{dsolve} routine in Maple [42] it can be seen that Equation (6.26) has the solution
\[
N_{G_1}(t) = C_1 e^{(2^{\frac{1}{2}}-1)t} F_3 \left( [-\alpha, -\alpha, -\alpha], \left[ 1 - 2\alpha, 1 + (1 - i)\alpha, 1 + (1 - i)\alpha \right], \Theta(t) \right) \\
+ C_2 e^{-(2^{\frac{1}{2}}+1)t} F_3 \left( [\alpha, \alpha, \alpha], \left[ 1 + 2\alpha, 1 + (1 + i)\alpha, 1 + (1 + i)\alpha \right], \Theta(t) \right) \\
+ C_3 e^{(2^{\frac{1}{2}}+1)t} F_3 \left( [-\alpha, -\alpha, -\alpha], \left[ 1 - 2\alpha, 1 - (1 - i)\alpha, 1 - (1 - i)\alpha \right], \Theta(t) \right) \\
+ C_4 e^{-(2^{\frac{1}{2}}+1)t} F_3 \left( [\alpha, \alpha, \alpha], \left[ 1 + 2\alpha, 1 + (1 + i)\alpha, 1 + (1 + i)\alpha \right], \Theta(t) \right),
\] (6.27)

where \( C_i \) for \( i \in \{1, 2, 3, 4\} \) are constants to be determined from imposing the boundary conditions, \( \alpha = \frac{2^{\frac{1}{2}}}{\lambda} \), \( \Theta(t) \) is the auxiliary function defined as
\[
\Theta(t) = \frac{D_1 e^{-\lambda t}}{\lambda},
\] (6.28)
and \( pF_q(a, b, z) \) is a generalised hypergeometric function\(^2\), with \( a \) and \( b \) being lists containing \( p \) and \( q \) entries respectively. The function \( pF_q(a, b, z) \) is defined as
\[
pF_q(a, b, z) = \sum_{n=0}^{\infty} \frac{z^n}{n!} \prod_{i=1}^{p} \frac{\Gamma(a_i+n)}{\Gamma(a_i)} \prod_{j=1}^{q} \frac{\Gamma(b_j+n)}{\Gamma(b_j)},
\] (6.29)

Despite Equation (6.26) having an exact analytic solution this does not lend itself readily to analysis. When all the rate constants \( k \) are equal an exact solution exists, generally this is not the case and as such an exact solution may not easily be found. It is therefore desirable to obtain an adequate approximation which is more amenable to analysis. To determine the form such an approximation may take it is helpful to first consider a simpler second order ODE of a similar form.

\(^2\)Further details about hypergeometric functions may be found in [63].
6.2 The Effect of a Cytotoxic Drug Interaction on A Population of Cells

**Approximation to a Second Order ODE**

Consider the second order ODE

\[
\frac{d^2x(t)}{dt^2} + (2 + e^{-\lambda t}) \frac{dx(t)}{dt} + (1 - \lambda)e^{-\lambda t}x(t) = 0, \tag{6.30}
\]

on which appropriate boundary conditions may be imposed. For \(\lambda \neq 1\) Equation (6.30) has the unique solution

\[
x(t) = Ae^{e^{-\lambda t} - 2\lambda x(t)^2} \left( I_{\frac{\lambda t}{x(t)^2}}(\Xi(t)) + I_{\frac{2\lambda t}{x(t)^2}}(\Xi(t)) \right) \\
+ Be^{e^{-\lambda t} - 2\lambda x(t)^2} \left( K_{\frac{\lambda t}{x(t)^2}}(\Xi(t)) - K_{\frac{2\lambda t}{x(t)^2}}(\Xi(t)) \right), \tag{6.31}
\]

where \(A\) and \(B\) are constants, to be determined by imposing suitable boundary conditions, \(\Xi(t)\) is the auxiliary function defined as

\[
\Xi(t) = \frac{e^{-\lambda t}}{2\lambda}, \tag{6.32}
\]

and \(I_\alpha\) and \(K_\alpha\) are the modified Bessel functions of order \(\alpha\) of the first and second kind respectively. Although this solution is expressed in a different form to that of Equation (6.27) it should be noted that Bessel functions may be readily converted into hypergeometric functions. To obtain a full numerical solution with which an approximation may be compared the arbitrary Dirichlet boundary conditions \(x(0) = 0\) and \(x(\infty) = 1\) are imposed. Despite the original fourth order ODE given in Equation (6.26) being an initial value problem, Dirichlet boundary conditions are imposed on this second order ODE so that the solution remains bounded and as such approximations may be easily verified. Upon applying these boundary conditions and assigning a value to \(\lambda\) the solution to Equation (6.30) given by (6.31) may now be obtained. Plots of this solution for the case \(\lambda = 5\) are shown in Figures 6.2 and 6.3. An approximation for the solution can be found by applying a change of variables. Let

\[
\tilde{t} = \Xi(t), \tag{6.33}
\]

which, upon substitution into Equation (6.30) gives

\[
\lambda \tilde{t}^2 \frac{d^2x(\tilde{t})}{d\tilde{t}^2} - (2 + 2\lambda \tilde{t} - \lambda) \frac{dx(\tilde{t})}{d\tilde{t}} + 2(1 - \lambda)x(\tilde{t}) = 0. \tag{6.34}
\]
6.2 The Effect of a Cytotoxic Drug Interaction on A Population of Cells

Figure 6.2: Plot of the Analytical Solution for the Second Order ODE Given in Equation (6.30) for $t \in [0, 5]$ and $\lambda = 5$.

For ease of later calculations it is helpful to multiply Equation (6.34) by $\frac{\dot{t}}{\lambda}$ to give

$$\dot{t}^2 \frac{d^2 x(\dot{t})}{dt^2} - \left(\frac{2\dot{t}}{\lambda} + 2\dot{t}^2 - \ddot{t}\right) \frac{dx(\dot{t})}{dt} (\dot{t}) + 2\dot{t} \left(\frac{1}{\lambda} - 1\right) x(\dot{t}) = 0. \quad (6.35)$$

This equation is singular at the point $\ddot{t} = 0$. To determine the nature of this singularity it is noted that Equation (6.35) is now of the form

$$P(\dot{t}) \frac{d^2 x(\dot{t})}{dt^2} + Q(\dot{t}) \frac{dx(\dot{t})}{dt} + R(\dot{t}) x(\dot{t}) = 0, \quad (6.36)$$

where

$$P(\dot{t}) = \dot{t}^2,$$

$$Q(\dot{t}) = -\left(\frac{2\dot{t}}{\lambda} + 2\dot{t}^2 - \ddot{t}\right), \quad (6.37)$$

$$R(\dot{t}) = 2\dot{t} \left(\frac{1}{\lambda} - 1\right).$$
Figure 6.3: Plot of the Analytical Solution for the Second Order ODE Given in Equation (6.30) for $t \in [0, 1]$ and $\lambda = 5$.

For a regular singularity $\lim_{t \to 0} \frac{\tilde{t}Q(\tilde{t})}{P(\tilde{t})}$ and $\lim_{t \to 0} \frac{\tilde{t}^2 R(\tilde{t})}{P(\tilde{t})}$ must both remain finite. From the values of $P(\tilde{t})$, $Q(\tilde{t})$ and $R(\tilde{t})$ given in Equations (6.37) it can be seen that

$$\lim_{t \to 0} \frac{\tilde{t}Q(\tilde{t})}{P(\tilde{t})} = \lim_{t \to 0} \frac{-\tilde{t} \left( \frac{2\tilde{t}}{\lambda} + 2t^2 - \tilde{t} \right)}{t^2},$$

$$= \lim_{t \to 0} - \left( \frac{2}{\lambda} + 2t - 1 \right),$$

$$= 1 - \frac{2}{\lambda},$$

and

$$\lim_{t \to 0} \frac{\tilde{t}^2 R(\tilde{t})}{P(\tilde{t})} = \lim_{t \to 0} \frac{\tilde{t}^2 \left( \frac{\tilde{t}}{\lambda} - 1 \right)}{t^2},$$

$$= \lim_{t \to 0} 2\tilde{t} \left( \frac{1}{\lambda} - 1 \right),$$

$$= 0.$$
Thus the singularity at $\tilde{t} = 0$ is regular. Because the singularity is regular, Equation (6.35) may be solved using Frobenius’ Method.

The first step in this method is to make the ansatz

$$x(\tilde{t}) = \tilde{t}^r \sum_{n=0}^{\infty} a_n \tilde{t}^n,$$

$$= \sum_{n=0}^{\infty} a_n \tilde{t}^{n+r},$$

where $a_0 \neq 0$. If $a_0 = 0$ and the first non-zero $a$ is $a_m$ then $r$ may be replaced by $r + m$ and consequently $a_m$ may be replaced by $a_0$. Clearly

$$\frac{dx(\tilde{t})}{d\tilde{t}} = \sum_{n=0}^{\infty} a_n \Phi_0^n \tilde{t}^{n-1},$$

(6.38)

and

$$\frac{d^2 x(\tilde{t})}{d\tilde{t}^2} = \sum_{n=0}^{\infty} a_n \Phi_0^n \Phi_{-1}^n \tilde{t}^{n-2},$$

(6.39)

where $\Phi_j^n$ is the auxiliary function defined as

$$\Phi_j^n = n + r + j.$$ 

(6.40)

Substituting these expressions for $x(\tilde{t})$ and its derivatives into Equation (6.35) yields

$$\sum_{n=0}^{\infty} a_n \Phi_0^n \Phi_{-1}^n \tilde{t}^n + \left(1 - \frac{2}{\lambda}\right) \sum_{n=0}^{\infty} a_n \Phi_0^n \tilde{t}^{n+1}$$

$$- 2 \sum_{n=0}^{\infty} a_n \Phi_0^n \tilde{t}^{n+1} + 2 \left(\frac{1}{\lambda} - 1\right) \sum_{n=0}^{\infty} a_n \tilde{t}^{n+1} = 0.$$ 

(6.41)

The last two summations in Equation (6.41) may be expressed as

$$- 2 \sum_{n=1}^{\infty} a_{n-1} \Phi_{-1}^n \tilde{t}^n,$$

(6.42)

and

$$2 \left(\frac{1}{\lambda} - 1\right) \sum_{n=1}^{\infty} a_{n-1} \tilde{t}^n,$$

(6.43)
respectively. Thus, Equation (6.41) may be re-written as
\[
a_0 \left[ \Phi_0^0 \Phi_{-1}^0 + \Phi_0^0 \left( 1 - \frac{2}{\lambda} \right) \right] \hat{t}^{\Phi_0^0} + \sum_{n=1}^{\infty} \left\{ \left[ \Phi_0^n \Phi_{-1}^n + \left( 1 - \frac{2}{\lambda} \right) \right] a_n + \left[ 2 \left( \frac{1}{\lambda} - 1 \right) - 2 \Phi_{-1}^n \right] a_{n-1} \right\} \hat{t}^{\Phi_n^0} = 0,
\]

i.e.
\[
a_0 \left[ r \left( r - \frac{2}{\lambda} \right) \right] \hat{t}^r + \sum_{n=1}^{\infty} \left\{ \left[ \Phi_0^n \left( \Phi_{-\frac{2}{\lambda}}^n \right) \right] a_n + \left[ -2 \left( \Phi_{-\frac{2}{\lambda}}^n \right) \right] a_{n-1} \right\} \hat{t}^{\Phi_n^0} = 0.
\]

For Equation (6.45) to be satisfied the coefficient for each power of \( \hat{t} \) must equal zero. Firstly, the coefficient of the \( \hat{t}^r \) term is considered,
\[
a_0 \left[ r(r - 1) + r \left( 1 - \frac{2}{\lambda} \right) \right] = 0.
\]

Since \( a_0 \neq 0 \) it is clear \( r = 0 \) or \( r = \frac{2}{\lambda} \). Equation (6.35) is linear in \( x(\hat{t}) \) therefore the general solution will be given by
\[
x(\hat{t}) = c_1 x_1(\hat{t}) + c_2 x_2(\hat{t}),
\]

where \( x_1(\hat{t}) \) and \( x_2(\hat{t}) \) are two linearly independent solutions and \( c_1 \) and \( c_2 \) are constants to be determined using the boundary conditions. The two values of \( r \) which satisfy Equation (6.46) produce two linearly independent solutions, therefore let \( x_1(\hat{t}) \) and \( x_2(\hat{t}) \) correspond to \( r = 0 \) and \( r = \frac{2}{\lambda} \) respectively. For the remaining coefficients, \( a_n \) and \( A_n \) for \( n \geq 1 \), to be zero
\[
\sum_{n=1}^{\infty} \left\{ \left[ n \left( n - \frac{2}{\lambda} \right) \right] a_n + \left[ -2 \left( n - \frac{1}{\lambda} \right) \right] a_{n-1} \right\} \hat{t}^n = 0,
\]

and
\[
\hat{t}^2 \left( A_0 + \sum_{n=1}^{\infty} \left\{ \left[ \left( n + \frac{2}{\lambda} \right) \left( n + \frac{2}{\lambda} - \frac{2}{\lambda} \right) \right] A_n + \left[ -2 \left( n + \frac{2}{\lambda} - \frac{1}{\lambda} \right) \right] A_{n-1} \right\} \hat{t}^n \right) = 0.
\]
6.2 The Effect of a Cytotoxic Drug Interaction on A Population of Cells

i.e.

\[
\hat{\xi}^2 \left( A_0 + \sum_{n=1}^{\infty} \left\{ \left[ n \left( n + \frac{2}{\lambda} \right) \right] A_n + \left[ -2 \left( n + \frac{1}{\lambda} \right) \right] A_{n-1} \right\} \hat{\xi}^n \right) = 0. \tag{6.50}
\]

Thus for \( a_n \) and \( A_n \), \( n \geq 1 \), to be zero the recurrence relations

\[
a_n = \frac{2 \left( n - \frac{1}{\lambda} \right)}{n \left( n - \frac{2}{\lambda} \right)} a_{n-1}, \tag{6.51}
\]

and

\[
A_n = \frac{2 \left( n + \frac{1}{\lambda} \right)}{n \left( n + \frac{2}{\lambda} \right)} A_{n-1}, \tag{6.52}
\]

must be satisfied.

The auxiliary function \( \xi_i^j \) is introduced, where

\[
\xi_i^j = i + \frac{j}{\lambda}. \tag{6.53}
\]

The first three terms in the recurrence relation given by Equation (6.51) are

\[
a_1 = \frac{2\xi_1^{-1}}{\xi_1} a_0, \tag{6.54}
\]

\[
a_2 = \frac{\xi_2^{-1}}{\xi_2} a_1, \tag{6.55}
\]

and

\[
a_3 = \frac{2\xi_3^{-1}}{3\xi_3^2} a_2, \tag{6.56}
\]

Since \( \frac{\Gamma(n+1)}{\Gamma(n)} = n \), it may be shown that \( a_n \) may be expressed using the \( \Gamma \) notation as

\[
a_n = \frac{2^n \Gamma \left( \xi_1^{-2} \right) \Gamma \left( n + \xi_1^{-1} \right)}{n! \Gamma \left( n + \xi_1^{-2} \right) \Gamma \left( \xi_1^{-1} \right)} a_0. \tag{6.57}
\]
6.2 The Effect of a Cytotoxic Drug Interaction on A Population of Cells

In a similar way it is possible to express $A_n$ as

$$A_n = \frac{2^n \Gamma (\xi_1^2) \Gamma (n + \xi_1^2)}{n! \Gamma (n + \xi_1^2) \Gamma (\xi_1^2)} A_0.$$  (6.58)

Thus, the expressions for $x_1(\hat{t})$ and $x_2(\hat{t})$ may now be written as

$$x_1(\hat{t}) = a_0 + \sum_{n=1}^{\infty} \left\{ \frac{2^n \Gamma (\xi_1^{-2}) \Gamma (n + \xi_1^{-1})}{n! \Gamma (n + \xi_1^{-2}) \Gamma (\xi_1^{-1})} \right\} a_0 \hat{t}^n,$$

$$= a_0 \left( 1 + \sum_{n=1}^{\infty} \left\{ \frac{2^n \Gamma (\xi_1^{-2}) \Gamma (n + \xi_1^{-1})}{n! \Gamma (n + \xi_1^{-2}) \Gamma (\xi_1^{-1})} \right\} \hat{t}^n \right),$$  (6.59)

and

$$x_2(\hat{t}) = \hat{t}^2 \left( a_0 + \sum_{n=1}^{\infty} \left\{ \frac{2^n \Gamma (\xi_1^2) \Gamma (n + \xi_1^2)}{n! \Gamma (n + \xi_1^2) \Gamma (\xi_1^2)} \right\} A_0 \hat{t}^n \right),$$

$$= A_0 \hat{t}^2 \left( 1 + \sum_{n=1}^{\infty} \left\{ \frac{2^n \Gamma (\xi_1^2) \Gamma (n + \xi_1^2)}{n! \Gamma (n + \xi_1^2) \Gamma (\xi_1^2)} \right\} \hat{t}^n \right),$$  (6.60)

respectively.

Hence, the complete solution is given by

$$x(\hat{t}) = C_1 \left( 1 + \sum_{n=1}^{\infty} \left\{ \frac{2^n \Gamma (\xi_1^{-2}) \Gamma (n + \xi_1^{-1})}{n! \Gamma (n + \xi_1^{-2}) \Gamma (\xi_1^{-1})} \right\} \hat{t}^n \right)$$

$$+ C_2 \hat{t}^2 \left( 1 + \sum_{n=1}^{\infty} \left\{ \frac{2^n \Gamma (\xi_1^2) \Gamma (n + \xi_1^2)}{n! \Gamma (n + \xi_1^2) \Gamma (\xi_1^2)} \right\} \hat{t}^n \right),$$  (6.61)

where $C_1$ and $C_2$ are constants incorporating $a_0$ and $A_0$, respectively. To check the convergence of the two infinite sums in this expression the ratio test is implemented. For

$$\sum_{n=1}^{\infty} \left\{ \frac{2^n \Gamma (\xi_1^{-2}) \Gamma (n + \xi_1^{-1})}{n! \Gamma (n + \xi_1^{-2}) \Gamma (\xi_1^{-1})} \right\} \hat{t}^n,$$  (6.62)
it can be seen the limit of the ratio of two successive terms is given by

\[
L = \lim_{n \to \infty} \left| \frac{\frac{\Gamma(n + \xi^{-1}_2) \Gamma(n + \xi^{-2}_1)}{\Gamma(n + \xi^{-2}_1) \Gamma(n + \xi^{-1}_1)}}{\frac{2^n \Gamma(n + \xi^{-1}_1) \Gamma(n + \xi^{-1}_2)}{n! \Gamma(n + \xi^{-2}_1) \Gamma(n + \xi^{-1}_2)} \tilde{t}^{n+1}} \right|
\]

\[
= \lim_{n \to \infty} \left| \frac{2 \Gamma(n + \xi^{-1}_1) \Gamma(n + \xi^{-1}_2)}{(n + 1) \Gamma(n + \xi^{-1}_1) \Gamma(n + \xi^{-1}_2)} \tilde{t}^{n+1} \right|
\]

\[
= \lim_{n \to \infty} \left| \frac{2 (n + \xi^{-1}_1) \Gamma(n + \xi^{-1}_1) \Gamma(n + \xi^{-1}_2)}{(n + 1) \Gamma(n + \xi^{-1}_1) \Gamma(n + \xi^{-1}_2)} \tilde{t}^{n+1} \right|
\]

\[
= 0.
\]  

(6.63)

Since \( L < 1 \) the series converges absolutely for all \( \tilde{t} \).

Similarly, for

\[
\sum_{n=1}^{\infty} \left\{ \frac{2^n \Gamma(n + \xi^{-1}_1) \Gamma(n + \xi^{-1}_2)}{n! \Gamma(n + \xi^{-2}_1) \Gamma(n + \xi^{-1}_2)} \tilde{t}^{n+1} \right\}
\]

it can be seen the limit of the ratio of two successive terms is given by

\[
L = \lim_{n \to \infty} \left| \frac{\frac{\Gamma(n + \xi^{-1}_1) \Gamma(n + \xi^{-1}_2)}{\Gamma(n + \xi^{-2}_1) \Gamma(n + \xi^{-1}_2)}}{\frac{2^n \Gamma(n + \xi^{-1}_1) \Gamma(n + \xi^{-1}_2)}{n! \Gamma(n + \xi^{-2}_1) \Gamma(n + \xi^{-1}_2)} \tilde{t}^{n+1}} \right|
\]

\[
= \lim_{n \to \infty} \left| \frac{2 \Gamma(n + \xi^{-1}_1) \Gamma(n + \xi^{-1}_2)}{(n + 1) \Gamma(n + \xi^{-1}_1) \Gamma(n + \xi^{-1}_2)} \tilde{t}^{n+1} \right|
\]

\[
= \lim_{n \to \infty} \left| \frac{2 (n + \xi^{-1}_1) \Gamma(n + \xi^{-1}_1) \Gamma(n + \xi^{-1}_2)}{(n + 1) \Gamma(n + \xi^{-1}_1) \Gamma(n + \xi^{-1}_2)} \tilde{t}^{n+1} \right|
\]

\[
= 0.
\]  

(6.64)

Hence this series also converges absolutely for all \( \tilde{t} \). Since the expression for \( x(t) \) given by Equation (6.61) converges it is possible to obtain an approximation by truncating the infinite series. For small \( \tilde{t} \) assume all terms greater than \( \tilde{t}^m \) may be ignored, \( x(\tilde{t}) \) may be written as

\[
x(\tilde{t}) = P(\tilde{t}) + \mathcal{O}(\tilde{t}^{m+\frac{2}{3}}),
\]  

(6.66)
where

\[
P(\tilde{t}) = C_1 \left( 1 + \sum_{n=1}^{m} \left\{ \frac{2^n \Gamma \left( \xi_1^2 \right) \Gamma \left( n + \xi_1^1 \right)}{n!\Gamma \left( n + \xi_1^1 \right) \Gamma \left( \xi_1^1 \right)} \right\} \tilde{t}^n \right) \\
+ C_2 \tilde{t}^2 \left( 1 + \sum_{n=1}^{m-1} \left\{ \frac{2^n \Gamma \left( \xi_1^2 \right) \Gamma \left( n + \xi_1^1 \right)}{n!\Gamma \left( n + \xi_1^1 \right) \Gamma \left( \xi_1^1 \right)} \right\} \tilde{t}^n \right). \tag{6.67}
\]

To compare this approximation with the solution given in Equation (6.31), the values of

two and five are assigned to \( m \) and \( \lambda \) respectively. It should be noted this is the same

value of \( \lambda \) used in Figures 6.2 and 6.3. For this \( x(\tilde{t}) \) may be written as

\[
x(\tilde{t}) = P(\tilde{t}) + O(\tilde{t}^{12}), \tag{6.68}
\]

where

\[
P(\tilde{t}) = C_1 \left( 1 + \sum_{n=1}^{2} \left\{ \frac{2^n \Gamma \left( \frac{3}{2} \right) \Gamma \left( n + \frac{4}{2} \right)}{n!\Gamma \left( n + \frac{4}{2} \right) \Gamma \left( \frac{3}{2} \right)} \right\} \tilde{t}^n \right) \\
+ C_2 \tilde{t}^2 \left( 1 + \sum_{n=1}^{1} \left\{ \frac{2^n \Gamma \left( \frac{7}{2} \right) \Gamma \left( n + \frac{5}{2} \right)}{n!\Gamma \left( n + \frac{5}{2} \right) \Gamma \left( \frac{7}{2} \right)} \right\} \tilde{t}^n \right),
\]

\[
= C_1 \left( 1 + \frac{8}{3} \tilde{t} + 3\tilde{t}^2 \right) + C_2 \tilde{t}^2 \left( 1 + \frac{12}{7} \tilde{t} \right). \tag{6.69}
\]

When \( t = \infty, \tilde{t} = 0 \) and when \( t = 0, \tilde{t} = \frac{1}{2\lambda} = \frac{1}{10} \), therefore from the Dirichlet boundary conditions \( x(0) = 0 \) and \( x(\infty) = 1 \), we obtain \( P(0) = 1 \) and \( P(\frac{1}{10}) = 0 \). By setting

\( P(0) = 1 \) it is clear that

\[
C_1 = 1. \tag{6.70}
\]

Imposing the boundary condition \( P(\frac{1}{10}) = 0 \), together with \( C_1 = 1 \), yields

\[
C_2 = -\frac{2723}{2460} \approx 10^2, \approx -2.78. \tag{6.71}
\]

Thus, for small \( \tilde{t} \) a second order approximation of \( x(\tilde{t}) \) is given by

\[
x(\tilde{t}) = \left( 1 + \frac{8}{3} \tilde{t} + 3\tilde{t}^2 \right) - 2.78\tilde{t}^2 \left( 1 + \frac{12}{7} \tilde{t} \right). \tag{6.72}
\]
6.2 The Effect of a Cytotoxic Drug Interaction on A Population of Cells

By using the substitution given in Equation (6.33) this may be expressed in terms of the original variable, \( t \) as

\[
x(t) = \left( 1 + \frac{8}{3} \left( \frac{e^{-5t}}{10} \right) + 3 \left( \frac{e^{-5t}}{10} \right)^2 \right) - 2.78 \left( \frac{e^{-5t}}{10} \right)^{\frac{5}{2}} \left( 1 + \frac{12}{7} \left( \frac{e^{-5t}}{10} \right) \right).
\]

This approximation is plotted together with the actual solution in Figure 6.4.

![Figure 6.4: Plot Showing a Second Order Approximation Together with Actual Solution for the Second Order ODE Given in Equation (6.30).](image)

Since the analytical solution to Equation (6.30) is known it is possible to conduct a further check of this approximation. Further details of this can be found in Appendix E.

Motivated by the techniques used to solve Equation (6.30) the transformation

\[
\tilde{t} = \lambda \Theta(t),
\]

(6.74)
where \( \Theta(t) \) is defined in Equation (6.28), is applied to Equation (6.26), yielding

\[
\lambda^4 \frac{d^4 N_{G1}(\hat{t})}{dt^4} + \left(6\lambda^4 \hat{t}^3 - 4\lambda^3 \hat{t}^2 - \lambda^2 \hat{t}^2 \right) \frac{d^3 N_{G1}(\hat{t})}{dt^3} + \left(7\lambda^4 \hat{t}^2 - 12\lambda^3 \hat{t}^2 - 3\lambda^2 \hat{t}^2 + 6\lambda^2 \hat{t}^2 + 3\lambda^2 \hat{t}^2 \right) \frac{d^2 N_{G1}(\hat{t})}{dt^2} + \left(\lambda^4 \hat{t} - 4\lambda^3 \hat{t} - \lambda^2 \hat{t}^2 + 6\lambda^2 \hat{t} + 3\lambda^2 \hat{t}^2 - 4\lambda \hat{t} - 3\lambda \hat{t}^2 \right) \frac{d N_{G1}(\hat{t})}{dt} + (\hat{t} - 1) N_{G1}(\hat{t}) = 0.
\]  
(6.75)

Equation (6.75) is singular at the point \( \hat{t} = 0 \). Proceeding in a similar manner as before it is noted that Equation (6.75) is of the form

\[
P(\hat{t}) \frac{d^4 N_{G1}(\hat{t})}{dt^4} + Q(\hat{t}) \frac{d^3 N_{G1}(\hat{t})}{dt^3} + R(\hat{t}) \frac{d^2 N_{G1}(\hat{t})}{dt^2} + S(\hat{t}) \frac{d N_{G1}(\hat{t})}{dt} + T(\hat{t}) N_{G1}(\hat{t}) = 0, \quad (6.76)
\]

where

\[
P(\hat{t}) = \lambda^4 \hat{t}^4,
\]
(6.77a)

\[
Q(\hat{t}) = 6\lambda^4 \hat{t}^3 - 4\lambda^3 \hat{t}^3 - \lambda^2 \hat{t}^2,
\]
(6.77b)

\[
R(\hat{t}) = 7\lambda^4 \hat{t}^2 - 12\lambda^3 \hat{t}^2 - 3\lambda^2 \hat{t}^2 + 6\lambda^2 \hat{t}^2 + 3\lambda^2 \hat{t}^2,
\]
(6.77c)

\[
S(\hat{t}) = \lambda^4 \hat{t} - 4\lambda^3 \hat{t} - \lambda^2 \hat{t}^2 + 6\lambda^2 \hat{t} + 3\lambda^2 \hat{t}^2 - 4\lambda \hat{t} - 3\lambda \hat{t}^2,
\]
(6.77d)

and

\[
T(\hat{t}) = \hat{t} - 1.
\]
(6.77e)

For \( \hat{t} = 0 \) to be a regular singularity \( \lim_{\hat{t} \to 0} \frac{\hat{t} Q(\hat{t})}{P(\hat{t})}, \lim_{\hat{t} \to 0} \frac{\hat{t}^2 R(\hat{t})}{P(\hat{t})}, \lim_{\hat{t} \to 0} \frac{\hat{t}^3 S(\hat{t})}{P(\hat{t})} \) and \( \lim_{\hat{t} \to 0} \frac{\hat{t}^4 T(\hat{t})}{P(\hat{t})} \)
must all remain finite. Using the expressions from Equations (6.77) yields

\[
\lim_{t \to 0} \frac{\ddot{i}Q(t)}{P(t)} = \lim_{t \to 0} \frac{\ddot{i}(6\lambda^4\dddot{t}^3 - 4\lambda^3\dddot{t}^2 - \lambda^2\dddot{t})}{\lambda^4t^4},
\]

\[
= \lim_{t \to 0} 6 - \left( \frac{4 + \dddot{t}}{\lambda} \right),
\]

\[
= \frac{2}{\lambda} (3\lambda - 2),
\]

(6.78)

and

\[
\lim_{t \to 0} \frac{\dddot{i}^2R(t)}{P(t)} = \lim_{t \to 0} \frac{\dddot{i}^2(7\lambda^4\dddot{t}^2 - 12\lambda^3\dddot{t}^2 - 3\lambda^3\dddot{t}^2 + 6\lambda^2\dddot{t}^2 + 3\lambda^2\dddot{t})}{\lambda^4t^4},
\]

\[
= \lim_{t \to 0} 7 - \left( \frac{12 + 3\dddot{t}}{\lambda} \right) + \left( \frac{6 + 3\dddot{t}}{\lambda^2} \right),
\]

\[
= \frac{1}{\lambda^2} (7\lambda^2 - 12\lambda + 6),
\]

(6.79)

and

\[
\lim_{t \to 0} \frac{\dddot{i}^3S(t)}{P(t)} = \lim_{t \to 0} \frac{\dddot{i}^3(\lambda^4\dddot{t}^4 - 4\lambda^3\dddot{t}^4 - \lambda^3\dddot{t}^2 + 6\lambda^2\dddot{t}^2 + 3\lambda^2\dddot{t}^2 - 4\lambda\dddot{t} - 3\lambda^2\dddot{t})}{\lambda^4t^4},
\]

\[
= \lim_{t \to 0} 1 - \left( \frac{4 + \dddot{t}}{\lambda} \right) + \left( \frac{6 + 3\dddot{t}}{\lambda^2} \right) - \left( \frac{4 + 3\dddot{t}}{\lambda^3} \right),
\]

\[
= \frac{1}{\lambda^3} (\lambda^3 - 4\lambda^2 + 6\lambda - 4),
\]

(6.80)

and

\[
\lim_{t \to 0} \frac{\dddot{i}^4T(t)}{P(t)} = \lim_{t \to 0} \frac{\dddot{i}^4(\dddot{t} - 1)}{\lambda^4t^4},
\]

\[
= \lim_{t \to 0} \frac{\dddot{t} - 1}{\lambda^3},
\]

\[
= \frac{-1}{\lambda^3}.
\]

(6.81)
Hence the singularity at \( \tilde{t} = 0 \) is regular, therefore Equation (6.75) may be solved using Frobenius’ Method, using the ansatz

\[
N_{G1}(\tilde{t}) = \tilde{t}^r \sum_{n=0}^{\infty} a_n \tilde{t}^n,
\]

\[
= \sum_{n=0}^{\infty} a_n \tilde{t}^{\Phi_0^n},
\]

(6.82)

where \( \Phi_0^n \) is defined in Equation (6.40) and \( a_0 \neq 0 \). The first four derivatives of \( N_{G1}(\tilde{t}) \) with respect to \( \tilde{t} \) are given by

\[
\frac{dN_{G1}(\tilde{t})}{d\tilde{t}} = \sum_{n=0}^{\infty} a_n \Phi_0^n \tilde{t}^{\Phi_0^n - 1},
\]

(6.83a)

\[
\frac{d^2N_{G1}(\tilde{t})}{d\tilde{t}^2} = \sum_{n=0}^{\infty} a_n \Phi_0^n \Phi_{-1}^n \tilde{t}^{\Phi_0^n - 2},
\]

(6.83b)

\[
\frac{d^3N_{G1}(\tilde{t})}{d\tilde{t}^3} = \sum_{n=0}^{\infty} a_n \Phi_0^n \Phi_{-1}^n \Phi_{-2}^n \tilde{t}^{\Phi_0^n - 3},
\]

(6.83c)

and

\[
\frac{d^4N_{G1}(\tilde{t})}{d\tilde{t}^4} = \sum_{n=0}^{\infty} a_n \Phi_0^n \Phi_{-1}^n \Phi_{-2}^n \Phi_{-3}^n \tilde{t}^{\Phi_0^n - 4}.
\]

(6.83d)

Substituting these expressions for \( N_{G1}(\tilde{t}) \), and its derivatives into Equation (6.75) gives

\[
\lambda^4 \sum_{n=0}^{\infty} a_n \Phi_0^n \Phi_{-1}^n \Phi_{-2}^n \Phi_{-3}^n \tilde{t}^{\Phi_0^n} + (6\lambda^4 - 4\lambda^3) \sum_{n=0}^{\infty} a_n \Phi_0^n \Phi_{-1}^n \Phi_{-2}^n \tilde{t}^{\Phi_0^n}
\]

\[
- \lambda^3 \sum_{n=0}^{\infty} a_n \Phi_0^n \Phi_{-1}^n \Phi_{-2}^n \tilde{t}^{\Phi_0^n} + (7\lambda^4 - 12\lambda^3 + 6\lambda^2) \sum_{n=0}^{\infty} a_n \Phi_0^n \Phi_{-1}^n \tilde{t}^{\Phi_0^n}
\]

\[
+ 3 (\lambda^2 - \lambda^3) \sum_{n=0}^{\infty} a_n \Phi_0^n \Phi_{-1}^n \tilde{t}^{\Phi_0^n} + (\lambda^4 - 4\lambda^3 + 6\lambda^2 - 4\lambda) \sum_{n=0}^{\infty} a_n \Phi_0^n \tilde{t}^{\Phi_0^n}
\]

\[
+ (3\lambda^2 - 3\lambda - \lambda^3) \sum_{n=0}^{\infty} a_n \Phi_0^n \tilde{t}^{\Phi_0^n} - \sum_{n=0}^{\infty} a_n \tilde{t}^{\Phi_0^n} + \sum_{n=0}^{\infty} a_n \tilde{t}^{\Phi_0^n} = 0,
\]

(6.84)
which may be rewritten as
\[
\begin{align*}
\lambda^4 & \sum_{n=0}^{\infty} a_n \Phi_0^n \Phi_{-1}^n \Phi_{-2}^n \Phi_{-3}^n \tilde{r}^{0^n} + (6\lambda^4 - 4\lambda^3) \sum_{n=0}^{\infty} a_n \Phi_0^n \Phi_{-1}^n \Phi_{-2}^n \tilde{r}^{0^n} \\
& - \lambda^3 \sum_{n=1}^{\infty} a_{n-1} \Phi_{-1}^n \Phi_{-2}^n \Phi_{-3}^n \tilde{r}^{0^n} + (7\lambda^4 - 12\lambda^3 + 6\lambda^2) \sum_{n=0}^{\infty} a_n \Phi_0^n \Phi_{-1}^n \tilde{r}^{0^n} \\
& + 3 (\lambda^2 - \lambda^3) \sum_{n=1}^{\infty} a_{n-1} \Phi_{-1}^n \Phi_{-2}^n \tilde{r}^{0^n} + (\lambda^4 - 4\lambda^3 + 6\lambda^2 - 4\lambda) \sum_{n=0}^{\infty} a_n \Phi_0^n \tilde{r}^{0^n} \\
& + (3\lambda^2 - 3\lambda - \lambda^3) \sum_{n=1}^{\infty} a_{n-1} \Phi_{-1}^n \tilde{r}^{0^n} - \sum_{n=0}^{\infty} a_n \tilde{r}^{0^n} + \sum_{n=1}^{\infty} a_{n-1} \tilde{r}^{0^n} = 0, \tag{6.85}
\end{align*}
\]
i.e.
\[
\begin{align*}
a_0 \left[ & \lambda^4 \Phi_0^0 \Phi_{-1}^0 \Phi_{-2}^0 \Phi_{-3}^0 + (6\lambda^4 - 4\lambda^3) \Phi_0^0 \Phi_{-1}^0 \Phi_{-2}^0 \\
& + (7\lambda^4 - 12\lambda^3 + 6\lambda^2) \Phi_0^0 \Phi_{-1}^0 + (\lambda^4 - 4\lambda^3 + 6\lambda^2 - 4\lambda) \Phi_0^0 - 1 \right] \tilde{r}^{0^n} \\
& + \sum_{n=1}^{\infty} \left\{ a_n \left[ \lambda^4 \Phi_0^n \Phi_{-1}^n \Phi_{-2}^n \Phi_{-3}^n + (6\lambda^4 - 4\lambda^3) \Phi_0^n \Phi_{-1}^n \Phi_{-2}^n \\
& + (7\lambda^4 - 12\lambda^3 + 6\lambda^2) \Phi_0^n \Phi_{-1}^n + (\lambda^4 - 4\lambda^3 + 6\lambda^2 - 4\lambda) \Phi_0^n - 1 \right] \tilde{r}^{0^n} \\
& + a_{n-1} \left[ -\lambda^3 \Phi_{-1}^n \Phi_{-2}^n \Phi_{-3}^n + 3(\lambda^2 - \lambda^3) \Phi_{-1}^n \Phi_{-2}^n \\
& + (3\lambda^2 - 3\lambda - \lambda^3) \Phi_{-1}^n + 1 \right] \tilde{r}^{0^n} = 0, \tag{6.86}
\end{align*}
\]
which upon simplifying becomes
\[
\begin{align*}
a_0 \left[ (\lambda r - 1)^4 - 2 \right] \tilde{r} \\
& + \sum_{n=1}^{\infty} \left\{ a_n \left[ (\Phi_{-1}^n \lambda - 1)^4 - 2 \right] - a_{n-1} \left[ (\Phi_{-1}^n \lambda - 1)^3 \right] \right\} \tilde{r}^{0^n} = 0. \tag{6.87}
\end{align*}
\]
If Equation (6.87) is to be satisfied then the coefficient for each power of \( \tilde{r} \) must equal zero. Considering the coefficient of the \( \tilde{r} \) term gives
\[
a_0 \left[ (\lambda r - 1)^4 - 2 \right] = 0. \tag{6.88}
\]
Since \( a_0 \neq 0 \) it is clear \( r \) satisfies the quartic equation

\[
(\lambda r - 1)^4 - 2 = 0. \tag{6.89}
\]

Therefore

\[
r = \frac{1 \pm 2^{\frac{1}{4}}}{\lambda}, \quad 1 \pm 2^{\frac{1}{4}}i. \tag{6.90}
\]

Since all the roots, \( r \), are distinct all four solutions are linearly independent. Equation (6.75) is linear in \( N_G(\bar{t}) \), therefore the general solution will be given by a linear combination of the four independent solutions corresponding to the four values of \( r \) satisfying Equation (6.89). Hence,

\[
N_G(\bar{t}) = c_1 x_1(\bar{t}) + c_2 x_2(\bar{t}) + c_3 x_3(\bar{t}) + c_4 x_4(\bar{t}) = 0, \tag{6.91}
\]

where \( x_j(\bar{t}) \) for \( j \in \{1, 2, 3, 4\} \) are the four linearly independent solutions corresponding to the four values of \( r \) satisfying Equation (6.89) and \( c_j(\bar{t}) \) for \( j \in \{1, 2, 3, 4\} \) are constants to be determined using the initial conditions. Unlike the analysis of the second order ODE, the recurrence relations and convergence of the four series resulting in the different \( r \) values are considered together. For the coefficients of \( \bar{t}^n \) for \( n \geq 1 \) to equal zero the recurrence relation

\[
a_n = \frac{(\Phi_n^0 \lambda - 1)^3}{((\Phi_n^0 \lambda - 1)^4 - 2)} a_{n-1}, \tag{6.92}
\]

must be satisfied. To check the resulting series converge the ratio test may be implemented. It can be seen that the ratio of two successive terms is given by

\[
L = \lim_{n \to \infty} \left| \frac{a_n \bar{t}^n}{a_{n-1} \bar{t}^{n-1}} \right|,
\]

\[
= \lim_{n \to \infty} \left| \frac{(\Phi_n^0 \lambda - 1)^3}{((\Phi_n^0 \lambda - 1)^4 - 2)} a_{n-1} \bar{t} \right|,
\]

\[
= \lim_{n \to \infty} \left| \frac{(\Phi_n^0 \lambda - 1)^3}{((\Phi_n^0 \lambda - 1)^4 - 2)} \right|,
\]

\[
= 0. \tag{6.93}
\]

Since \( L < 1 \) the series converges absolutely for all \( \bar{t} \). To obtain a first order approximation (in terms of \( \bar{t} \)), for the complete solution it is necessary to look at the real parts of the four
The Effect of a Cytotoxic Drug Interaction on A Population of Cells

\[ r \text{ values given in Equation (6.90)} \]. Since \( R_e(r) \geq 1 - 2^\frac{1}{r} > -1 \) it is necessary to calculate the solution up to terms containing \( \tilde{t}^{r+2} \) to obtain a first order approximation. Whilst it is not necessary to include the terms for \( n = 2 \) for all of the \( r \) to obtain a first order approximation they are included so that each series is truncated after the same number of terms. Thus, for small \( \tilde{t} \), \( N_{G1}(\tilde{t}) \) may be written as

\[
N_{G1}(\tilde{t}) = P(\tilde{t}) + \mathcal{O}(\tilde{t}^2),
\]

where

\[
P(\tilde{t}) = \sum_{j=1}^{4} a_{j_0} \tilde{t}^3 \left\{ 1 + \frac{(r_j \lambda - 1)^3}{(r_j \lambda - \lambda - 1)^4 - 2 \tilde{t}} + \frac{(r_j \lambda + \lambda - 1)^3(r_j \lambda - 1)^3}{(r_j \lambda + 2 \lambda - 1)^4 - 2 \tilde{t}} \right\},
\]

where the \( j \) correspond to the four different values of \( r \). It should be noted that the constants \( c_j \) have now been incorporated into the values of \( a_j \). In order to compare the approximation with the analytic solution given by Equation (6.27) suitable parameter values and initial conditions must be chosen. The parameters \( D_1 \) and \( \lambda \) are chosen to have the arbitrary values of one and two respectively. The initial conditions given in Equation (6.7) state the cell population density is the same for all four phases, therefore

\[
N_j(0) = 1 \text{ for } j \in \{G_1, S, G_2, M\}.
\]

These initial conditions have been chosen to avoid any problems which might arise by using the unrealistic scenario of all cells initially being in one phase. Using Equations (6.23), (6.24) and (6.25) these initial conditions can readily be transformed into the Cauchy initial conditions

\[
N_{G1}(0) = 1,
\]

\[
\frac{dN_{G1}(t)}{dt} \bigg|_{t=0} = 1,
\]

\[
\frac{d^2N_{G1}(t)}{dt^2} \bigg|_{t=0} = -1,
\]

and

\[
\frac{d^3N_{G1}(t)}{dt^3} \bigg|_{t=0} = 1,
\]

for Equation (6.26).
The full analytic solution is now given by

\[
N_{G_1}(t) = 1.1927 e^{(2^{\frac{1}{2}-1})t} F_3 \left(\left[-\alpha, -\alpha, -\alpha\right], [1 - 2\alpha, 1 + (-1 + i)\alpha, 1 - (1 + i)\alpha], \Theta(t) \right)
\]

\[- 0.9724 e^{-(2^{\frac{1}{2}+1})t} F_3 \left([\alpha, \alpha, \alpha], [1 + 2\alpha, 1 + (1 + i)\alpha, 1 + (1 - i)\alpha], \Theta(t) \right)\]

\[- (0.2521 + 0.2128i) e^{(2^{\frac{1}{2}i-1})t} F_3 \left(\left[-\alpha i, -\alpha i, -\alpha i\right], [1 - 2\alpha i, 1 + (1 - i)\alpha, 1 - (1 + i)\alpha], \Theta(t) \right)\]

\[- (0.2521 - 0.2128i) e^{-(2^{\frac{1}{2}i+1})t} F_3 \left([\alpha i, \alpha i, \alpha i], [1 + 2\alpha i, 1 + (1 + i)\alpha, 1 + (-1 + i)\alpha], \Theta(t) \right),\]

where \( \alpha = 2^{-\frac{3}{4}} \), and \( \Theta(t) \) is the auxiliary function defined as

\[
\Theta(t) = \frac{e^{-2t}}{2}.
\]

(6.99)

In order to find the first order approximation given by Equation (6.95) the initial conditions given in Equations (6.97) must be transformed to suitable boundary conditions for Equation (6.75). These correspond to the Cauchy boundary conditions

\[
N_{G_1}(1) = 1,
\]

(6.100a)

\[
\frac{dN_{G_1}(\tilde{t})}{dt} \bigg|_{\tilde{t}=1} = -\frac{1}{2},
\]

(6.100b)

\[
\frac{d^2 N_{G_1}(\tilde{t})}{dt^2} \bigg|_{\tilde{t}=1} = \frac{1}{4},
\]

(6.100c)

and

\[
\frac{d^3 N_{G_1}(\tilde{t})}{dt^3} \bigg|_{\tilde{t}=1} = -\frac{3}{8}.
\]

(6.100d)
Applying these boundary conditions to Equation (6.95) gives

\[ a_{10} = -0.2527 + 0.2192i, \]
\[ a_{20} = 1.1874, \]
\[ a_{30} = -0.2527 - 0.2192i, \]
\[ a_{40} = -0.9596, \]

where \( a_{j0}, j \in \{1, 2, 3, 4\} \), correspond to \( r_j \) where

\[ r_j = \frac{1 + 2^i j^2}{2}. \]  

(6.102)

Note, by using the substitution given in Equation (6.74), Equation (6.95) may be expressed in terms of the original variable, \( t \) as

\[
P(t) = \sum_{j=1}^{4} a_{j0} \left( e^{-2t} \right) r_j \left\{ 1 + \frac{(r_j \lambda - 1)^3}{(r_j \lambda + \lambda - 1)^4 - 2 (e^{-2t})} + \frac{(r_j \lambda + \lambda - 1)^3 (r_j \lambda - 1)^3}{((r_j \lambda + 2 \lambda - 1)^4 - 2)} \left\{ (r_j \lambda + \lambda - 1)^4 - 2 \right\} (e^{-2t})^2 \right\}.
\]

(6.103)

This approximation is plotted together with the actual solution in Figure 6.5. Since the analytical solution to Equation (6.26) is known it is possible to conduct a further check of this approximation. Further details of this can be found in Appendix E.

Whilst Figure 6.5 demonstrates that this technique for approximating the solution is in good agreement with the analytical solution further work is necessary to gain information about the behaviour of the whole population of cells.

For the general case, where \( \lambda \) and \( D_I \) are arbitrary, an approximation to the population of cells in the \( G_1 \) compartment is given by Equation (6.95), or in terms of the original
Figure 6.5: Plot Showing a First Order Approximation Together with Actual Solution for the Fourth Order ODE Given in Equation (6.26) with $D_I = 1$ and $\lambda = 2$.

time variable $t$

\[
N_{G1}(t) \approx P(t) = \sum_{j=1}^{4} a_{jo} (D_I e^{-\lambda t}) r_j \left\{ 1 + \frac{(r_j \lambda - 1)^3}{(r_j \lambda + \lambda - 1)^4 - 2} (D_I e^{-\lambda t}) \right. \\
+ \left. \frac{(r_j \lambda + \lambda - 1)^3 (r_j \lambda - 1)^3}{(r_j \lambda + 2\lambda - 1)^4 - 2} \{(r_j \lambda + \lambda - 1)^4 - 2\} (D_I e^{-\lambda t})^2 \right\}.
\] (6.104)

Using this approximation, together with its first derivative it is now possible to readily obtain an approximation for $N_M(t)$ by using Equation (6.22a). In turn, an approximation for $N_{G2}(t)$ may be found using this approximation for $N_M(t)$ and Equation (6.22d). Repeating the process once more allows an approximation to be found for $N_S(t)$ via Equation (6.22c). A plot for the total cell count, $N_T(t)$, for the parameters $D_I = 1$ and $\lambda = 2$ is shown in Figure 6.6. As can be seen from Figure 6.6 for the parameter values chosen the total number of cells is monotonically increasing, thus the effect of this drug is to only slow down the population growth and not reduce the total population.
To determine if it is possible to actually reduce the population of cells it is necessary to consider the equations governing $N_T(t)$. By combining Equations (6.22) it is possible to obtain

$$\frac{dN_T(t)}{dt} = N_M(t) - D_I e^{-\lambda} N_S(t). \quad (6.105)$$

If the total population is to decrease then $\frac{dN_T(t)}{dt}$ must be negative for some $t$. The drug concentration, and hence its effects on the population, are monotonically decreasing therefore if $\frac{dN_T(t)}{dt} < 0$ for some $t \geq 0$ then

$$\left. \frac{dN_T(t)}{dt} \right|_{t=0} < 0, \quad (6.106)$$

therefore

$$N_M(0) - D_I N_S(0) < 0. \quad (6.107)$$

From the initial conditions given in Equation (6.19) if Equation (6.107) is to be satisfied then

$$D_I > 1. \quad (6.108)$$
6.2 The Effect of a Cytotoxic Drug Interaction on A Population of Cells

Furthermore, if the population is initially in decline it is possible to find the time at which a minimum number of cells is present by finding when the derivative of \( N_T(t) \) is zero, i.e.

\[
N_M(t) - D_I e^{-\lambda t} N_S(t) = 0.
\]  

(6.109)

It is clear that this will be a minimum by considering the physical nature of the system. Any effects of the drug will diminish resulting in the population being able to recover. A plot for the total cell count, \( N_T(t) \), for the parameters \( D_I = 2 \) and \( \lambda = 2 \) is shown in Figure 6.7. For the parameter values chosen, Equation (6.109) has the solution \( t = 0.2 \),

![Figure 6.7: Plot Showing an Approximation to the Total Cell Population with Cytotoxic Effect when \( D_I = 2 \) and \( \lambda = 2 \).](image)

which also agrees with the plot shown in Figure 6.7.

By considering different values of \( \lambda \) for \( D_I \) fixed it can be shown that as \( \lambda \) decreases the time at which the turning point occurs increases and the minimum population decreases. Recall, \( \lambda \) is defined as \( \lambda = \frac{d_{out}}{k} \) which is the relative rate of the wash out of the drug \( d_{out} \) to the rate of cell progression through the cycle \( k \). Intuitively if the drug remains in the system for longer relative to the rate cells progress through the cycle more cells will be
effected hence the minimum will be lower. Also, if the relative wash out rate is lower then the time taken for the minimum to be achieved will be greater.

6.2.3 Case II : $\epsilon = \mathcal{O}(1)$

By definition $\epsilon = \frac{\delta a}{\tau}$, therefore for Case II to occur the total amount of drug used per unit time, $\delta a$ would need to be the same order as the average rate cells pass between the phases $k$. For most situations the amount of drug used per unit time is several orders of magnitude less than the rate cells pass between the phases. For Case II Equation (6.18) does not decouple from the system, This case is said to be the distinguished limit. Since no small parameter is present Equations (6.16) and (6.18) are now not able to be solved using the approach described in Case I. For this scenario alternative analytical or numerical techniques must be utilised. Since the purpose of this analysis is to develop semi-analytical techniques using an asymptotic approach this case has not been considered further.

6.2.4 Case III : $\epsilon \to \infty$

If $\epsilon \to \infty$, then since $N_T(0) = 4$ clearly $\epsilon N_T(t) \to \infty$ for some range of $t \geq 0$. Therefore only $\epsilon N_T(t) \to \infty$ will be considered for some range of $t \geq 0$. Let $\bar{\epsilon} = \frac{1}{\epsilon}$, so $\bar{\epsilon} \to 0$ as $\epsilon \to \infty$, and $\bar{t} = \epsilon t = \frac{1}{\bar{\epsilon}}$. Applying these rescalings to Equations (6.16) and (6.18) gives

$$\frac{1}{\bar{\epsilon}} \frac{dN_{G_1}(\bar{t})}{dt} = 2N_M(\bar{t}) - N_{G_1}(\bar{t}), \quad (6.110a)$$

$$\frac{1}{\bar{\epsilon}} \frac{dN_S(\bar{t})}{dt} = N_{G_1}(\bar{t}) - N_S(\bar{t}) - D(\bar{t})N_S(\bar{t}), \quad (6.110b)$$

$$\frac{1}{\bar{\epsilon}} \frac{dN_{G_2}(\bar{t})}{dt} = N_S(\bar{t}) - N_{G_2}(\bar{t}), \quad (6.110c)$$

$$\frac{1}{\bar{\epsilon}} \frac{dN_M(\bar{t})}{dt} = N_{G_2}(\bar{t}) - N_M(\bar{t}), \quad (6.110d)$$

and

$$\frac{dD(\bar{t})}{dt} = -\lambda \bar{\epsilon} D(\bar{t}) - N_T(\bar{t})D(\bar{t}). \quad (6.111)$$

Note the initial conditions remain unaffected by these rescalings, so

$$N_i(\bar{t}) = N_i(0) = 1 \text{ where } i \in \{G_1, S, G_2, M\}. \quad (6.112)$$
For the zeroth order approximation all $\tilde{c}$ terms may be ignored. Thus Equations (6.110) simplify to

\[
\frac{dN_{G_1}(\tilde{t})}{dt} = 0, \quad (6.113a)
\]

\[
\frac{dN_S(\tilde{t})}{dt} = 0, \quad (6.113b)
\]

\[
\frac{dN_{G_2}(\tilde{t})}{dt}(t) = 0, \quad (6.113c)
\]

\[
\frac{dN_M(\tilde{t})}{dt}(t) = 0. \quad (6.113d)
\]

Substituting $N_T(\tilde{t})$ with four in Equation (6.111) and neglecting the term multiplied by $\tilde{c}$ gives

\[
\frac{dD(\tilde{t})}{dt} = -4D(\tilde{t}). \quad (6.114)
\]

Hence,

\[
D(\tilde{t}) = D_Ie^{-4\tilde{t}}. \quad (6.115)
\]

Equation (6.115) may now be expressed in terms of the original time variable, $t$, as

\[
D(t) = D_Ie^{-4t}. \quad (6.116)
\]

But $\epsilon \to \infty$ therefore $D(t) \approx 0$. Thus, for the case $\epsilon \to \infty$ the drug has a negligible uptake and has a minimal effect on the population. This means the population of cells grows in the same manner as the untreated model described in Section 2.1.2.

6.3 The Effect of a Cytostatic Drug Interaction on A Population of Cells

If a drug has a purely cytostatic effect on a cell population then the functions $f_i(D(t))$, $i \in \{1, 2, 3, 4\}$ which represent the cytotoxic effect of the drug in the different phases are all zero. To allow ease of comparison between the modelling of a cytostatic and cytotoxic effects let the drugs affect on the proliferation be directly proportional to the drug concentration, this is a good approximation for some cytostatic drugs [21] and [14]. Therefore, let $P(D(t)) = 2(1 - eD(t))$ where $e$ can be thought of as the efficacy of the
drug. Since the average number of cells produced per division must remain positive it is clear $eD(t) \leq 1 \forall t \geq 0$. Equations (6.5) now simplify to

$$\frac{dN_{G1}(t)}{dt} = 2(1 - eD(t))kN_M(t) - kN_{G1}(t),$$

$$\frac{dN_S(t)}{dt} = kN_{G1}(t) - kN_S(t),$$

$$\frac{dN_{G2}(t)}{dt} = kN_S(t) - kN_{G2}(t),$$

$$\frac{dN_M(t)}{dt} = kN_{G2}(t) - kN_M(t).$$

(6.117)

Equations (6.4) and (6.117) together with suitable initial conditions now represent a simplified model with a cytostatic drug interaction. To complete the model initial conditions are required. For consistency the initial conditions chosen are the same as those used in Section 6.2, i.e.

$$N_i(0) = \frac{N_T(0)}{4} \text{ where } i \in \{G_1, S, G_2, M\},$$

(6.118)

and

$$D(0) = D_I.$$

(6.119)

### 6.3.1 Non-Dimensionalisation of the Equations

Proceeding in a similar manner as for the cytotoxic case the first step in the analysis of the system is to non-dimensionalise the equations. The variables $\tilde{t}$, $\tilde{D}(\tilde{t})$, $\tilde{N}_{G1}(\tilde{t})$, $\tilde{N}_S(\tilde{t})$, $\tilde{N}_{G2}(\tilde{t})$, $\tilde{N}_M(\tilde{t})$ and $\tilde{N}_T(\tilde{t})$ are introduced, their definitions being given in Equations (6.9). Substituting the non-dimensional variables into Equations (6.4) and (6.117) gives

$$a \frac{d\tilde{N}_{G1}(\tilde{t})}{d\tilde{t}} = 2(1 - ec\tilde{D}(\tilde{t}))ak\tilde{N}_M(\tilde{t}) - ak\tilde{N}_{G1}(\tilde{t}),$$

$$a \frac{d\tilde{N}_S(\tilde{t})}{d\tilde{t}} = ak\tilde{N}_{G1}(\tilde{t}) - ak\tilde{N}_S(\tilde{t}),$$

$$a \frac{d\tilde{N}_{G2}(\tilde{t})}{d\tilde{t}} = ak\tilde{N}_S(\tilde{t}) - ak\tilde{N}_{G2}(\tilde{t}),$$

$$a \frac{d\tilde{N}_M(\tilde{t})}{d\tilde{t}} = ak\tilde{N}_{G2}(\tilde{t}) - ak\tilde{N}_M(\tilde{t}),$$

(6.120)
and
\[
\frac{c}{b} \frac{d\tilde{D}(t)}{dt} = -\frac{cd_{out}\tilde{D}(t)}{b} - \delta ac\tilde{N}_T(t)\tilde{D}(t).
\] (6.121)

The corresponding initial conditions are now given by
\[
\tilde{N}_i(0) = \frac{\tilde{N}_T(0)}{4} \text{ where } i \in \{G_1, S, G_2, M\},
\] (6.122)

and
\[
\tilde{D}(0) = \tilde{D}_I \text{ where } \tilde{D}_I = \frac{D_I}{c}.
\] (6.123)

Equations (6.120) and (6.121) may be re-arranged to give, upon dropping the tilde notation,
\[
\frac{dN_{G_1}(t)}{dt} = 2(1 - ceD(t))bkN_M(t) - bkN_{G_1}(t),
\]
\[
\frac{dN_S(t)}{dt} = bkN_{G_1}(t) - bkN_S(t),
\]
\[
\frac{dN_{G_2}(t)}{dt} = bkN_S(t) - bkN_{G_2}(t),
\]
\[
\frac{dN_M(t)}{dt} = bkN_{G_2}(t) - bkN_M(t),
\] (6.124)

and
\[
\frac{dD(t)}{dt} = -bd_{out}D(t) - \delta abN_T(t)D(t).
\] (6.125)

The parameters \(b\) and \(c\) are now chosen as \(b = \frac{1}{2}\) and \(c = \frac{1}{6}\), thus, the system of equations now become
\[
\frac{dN_{G_1}(t)}{dt} = 2(1 - D(t))N_M(t) - N_{G_1}(t),
\] (6.126a)
\[
\frac{dN_S(t)}{dt} = N_{G_1}(t) - N_S(t),
\] (6.126b)
\[
\frac{dN_{G_2}(t)}{dt} = N_S(t) - N_{G_2}(t),
\] (6.126c)
\[
\frac{dN_M(t)}{dt} = N_{G_2}(t) - N_M(t),
\] (6.126d)

and
\[
\frac{dD(t)}{dt} = -\frac{d_{out}}{k} D(t) - \frac{\delta a}{k} N_T(t)D_n(t).
\] (6.127)
As in the cytotoxic non-dimensionalisation $a$ is chosen as $a = \frac{N_T(0)}{4}$ and the new parameters, $\lambda = \frac{\delta d a u}{k}$ and $\epsilon = \frac{\delta a}{k}$ are introduced. Thus Equation (6.127) may be rewritten as

$$\frac{dD(t)}{dt} = -\lambda D(t) - \epsilon N_T(t) D(t). \quad (6.128)$$

The non-dimensionalised form of the initial conditions for the cell population densities are now

$$N_i(0) = 1 \text{ where } i \in \{G_1, S, G_2, M\}. \quad (6.129)$$

Note, the non-dimensionalisation used in the cytotoxic and cytostatic cases is very similar with the only difference being the choice of the scaling parameter $c$. As before, there are three cases to consider

- **Case I**: $\epsilon \ll 1$,
- **Case II**: $\mathcal{O}(\epsilon) = 1$,
- **Case III**: $\epsilon \gg 1$.

### 6.3.2 Case I: $\epsilon \ll 1$

Proceeding in the same manner as for the cytotoxic model, it can be seen that for the zeroth order approximation Equation (6.128) decouples from the system and may be readily solved to give $D(t) = D_I e^{-\lambda t}$. Inserting this expression for $D(t)$ back into Equations (6.126) gives four first order ODEs which may now be converted into a single fourth order ODE for any of the dependent variables. For the cytostatic case the most convenient dependent variable to use for the single fourth order ODE is $N_M(t)$. Thus, Equations (6.126) may be expressed as

$$\frac{d^4 N_M(t)}{dt^4} + 4 \frac{d^3 N_M(t)}{dt^3} + 6 \frac{d^2 N_M(t)}{dt^2} + 4 \frac{dN_M(t)}{dt} - (1 - 2D_I e^{-\lambda t}) N_M(t) = 0. \quad (6.130)$$

As with the cytotoxic model, in order to aid construction of an approximate solution a change of variables is required. A suitable transformation is given by

$$\tilde{t} = \Theta(t), \quad (6.131)$$
where Θ(\(\hat{t}\)) is defined in Equation (6.28). Applying this transformation to Equation (6.130) leads to

\[
\lambda^4 \hat{t}^4 \frac{d^4 N_M(\hat{t})}{d\hat{t}^4} + \left(6\lambda^4 \hat{t}^3 - 4\lambda^3 \hat{t}^3\right) \frac{d^3 N_M(\hat{t})}{d\hat{t}^3} + \left(7\lambda^4 \hat{t}^2 - 12\lambda^3 \hat{t}^2 + 6\lambda^2 \hat{t}^2\right) \frac{d^2 N_M(\hat{t})}{d\hat{t}^2} + \left(\lambda^4 \hat{t} - 4\lambda^3 \hat{t} + 6\lambda^2 \hat{t} - 4\lambda\hat{t}\right) \frac{dN_M(\hat{t})}{d\hat{t}} + (2\lambda\hat{t} - 1) N_M(\hat{t}) = 0. \tag{6.132}
\]

This equation is singular at the point \(\hat{t} = 0\). To determine the nature of this singularity it is noted that Equation (6.132) is of the form given in Equation (6.76) where

\[
P(\hat{t}) = \lambda^4 \hat{t}^4, \tag{6.133a}
\]

\[
Q(\hat{t}) = 6\lambda^4 \hat{t}^3 - 4\lambda^3 \hat{t}^3, \tag{6.133b}
\]

\[
R(\hat{t}) = 7\lambda^4 \hat{t}^2 - 12\lambda^3 \hat{t}^2 + 6\lambda^2 \hat{t}^2, \tag{6.133c}
\]

\[
S(\hat{t}) = \lambda^4 \hat{t} - 4\lambda^3 \hat{t} + 6\lambda^2 \hat{t} - 4\lambda\hat{t}, \tag{6.133d}
\]

and

\[
T(\hat{t}) = 2\lambda\hat{t} - 1. \tag{6.133e}
\]

For \(\hat{t} = 0\) to be a regular singularity \(\lim_{\hat{t} \to 0} \frac{\hat{t}Q(\hat{t})}{P(\hat{t})}, \lim_{\hat{t} \to 0} \frac{\hat{t}^2 R(\hat{t})}{P(\hat{t})}, \lim_{\hat{t} \to 0} \frac{\hat{t}^3 S(\hat{t})}{P(\hat{t})}\) and \(\lim_{\hat{t} \to 0} \frac{\hat{t}^4 T(\hat{t})}{P(\hat{t})}\) must all remain finite. Using the values given in Equation (6.133) yields

\[
\lim_{\hat{t} \to 0} \frac{\hat{t}Q(\hat{t})}{P(\hat{t})} = \lim_{\hat{t} \to 0} \frac{\hat{t}(6\lambda^4 \hat{t}^3 - 4\lambda^3 \hat{t}^3)}{\lambda^4 \hat{t}^4},
\]

\[
= 6 - \frac{4}{\lambda},
\]

\[
= \frac{2}{\lambda}(3\lambda - 2), \tag{6.134}
\]
and

\[
\lim_{\tilde{t} \to 0} \frac{\tilde{t}^2 R(\tilde{t})}{P(\tilde{t})} = \lim_{\tilde{t} \to 0} \frac{\tilde{t}^2 (7\lambda^2 \tilde{t}^2 - 12\lambda \tilde{t}^2 + 6\lambda^2 \tilde{t}^2)}{\lambda^4 \tilde{t}^4},
\]

\[
= \frac{7 - 12 \lambda}{\lambda} + \frac{6}{\lambda^2},
\]

\[
= \frac{1}{\lambda^2} (7\lambda^2 - 12\lambda + 6),
\]

(6.135)

and

\[
\lim_{\tilde{t} \to 0} \frac{\tilde{t}^3 S(\tilde{t})}{P(\tilde{t})} = \lim_{\tilde{t} \to 0} \frac{\tilde{t}^3 (\lambda^4 \tilde{t} - 4\lambda^3 \tilde{t} + 6\lambda^2 \tilde{t} - 4\lambda \tilde{t})}{\lambda^4 \tilde{t}^4},
\]

\[
= 1 - \frac{4}{\lambda} + \frac{6}{\lambda^2} - \frac{4}{\lambda^3},
\]

\[
= \frac{1}{\lambda^3} (\lambda^3 - 4\lambda^2 + 6\lambda - 4),
\]

(6.136)

and

\[
\lim_{\tilde{t} \to 0} \frac{\tilde{t}^4 T(\tilde{t})}{P(\tilde{t})} = \lim_{\tilde{t} \to 0} \frac{\tilde{t}^4 (2\lambda \tilde{t} - 1)}{\lambda^4 \tilde{t}^4},
\]

\[
= \lim_{\tilde{t} \to 0} \frac{2\lambda \tilde{t} - 1}{\lambda^4},
\]

\[
= \frac{-1}{\lambda^4}.
\]

(6.137)

Hence the singularity at \( \tilde{t} = 0 \) is regular. Because the singularity is regular, Equation (6.132) may be solved using Frobenius' Method. Furthermore, it should be noted that despite Equation (6.75) and Equation (6.132) being different the limits obtained when determining the order of the poles at \( \tilde{t} = 0 \) for \( \frac{Q(\tilde{t})}{P(\tilde{t})}, \frac{R(\tilde{t})}{P(\tilde{t})}, \frac{S(\tilde{t})}{P(\tilde{t})} \) and \( \frac{T(\tilde{t})}{P(\tilde{t})} \), given by Equations (6.78)-(6.81) and Equations (6.134)-(6.137) are the same. Using the ansatz

\[
N_M(\tilde{t}) = \tilde{e}^r \sum_{n=0}^{\infty} a_n \tilde{t}^n,
\]

\[
= \sum_{n=0}^{\infty} a_n \tilde{t}^{n}, \quad (6.138)
\]
where \( a_0 \neq 0 \) and \( \Phi_i^j \) is defined in Equation (6.40), Equation (6.132) may be written as
\[
\lambda^4 \sum_{n=0}^{\infty} a_n \Phi_0^n \Phi_{-1}^n \Phi_{-2}^n \tilde{\Phi}_0^0 + \left( 6\lambda^4 - 4\lambda^3 \right) \sum_{n=0}^{\infty} a_n \Phi_0^n \Phi_{-1}^n \Phi_{-2}^n \tilde{\Phi}_0^0 \\
+ \left( 7\lambda^4 - 12\lambda^3 + 6\lambda^2 \right) \sum_{n=0}^{\infty} a_n \Phi_0^n \Phi_{-1}^n \tilde{\Phi}_0^0 + \left( \lambda^4 - 4\lambda^3 + 6\lambda^2 \right) \sum_{n=0}^{\infty} a_n \Phi_0^n \tilde{\Phi}_0^0 \\
- \sum_{n=0}^{\infty} a_n \tilde{\Phi}_0^n + 2\lambda \sum_{n=1}^{\infty} a_{n-1} \tilde{\Phi}_0^n = 0,
\]
which upon simplifying becomes
\[
 a_0 \left[ (\lambda r - 1)^4 - 2 \right] \tilde{r}^r + \sum_{n=1}^{\infty} \left\{ a_n \left[ (\Phi_0^n \lambda - 1)^4 - 2 \right] + 2\lambda a_{n-1} \right\} \tilde{\Phi}_0^n = 0. \tag{6.140}
\]
Equation (6.140) is very similar to (6.87), thus the general solution to Equation (6.132) will be given by a linear combination of the four independent solutions corresponding to the four values of \( r \) satisfying Equation (6.89). Hence,
\[
N_M(\tilde{t}) = c_1 y_1(\tilde{t}) + c_2 y_2(\tilde{t}) + c_3 y_3(\tilde{t}) + c_4 y_4(\tilde{t}) = 0, \tag{6.141}
\]
where \( y_j(\tilde{t}) \) for \( j \in \{1, 2, 3, 4\} \) are the four linearly independent solutions corresponding to the four values of \( r \) satisfying Equation (6.89) and \( c_j(\tilde{t}) \) for \( j \in \{1, 2, 3, 4\} \) are constants to be determined using the initial conditions. For the coefficients of \( \tilde{\Phi}_0^n \) for \( n \geq 1 \) to equal zero the recurrence relation
\[
 a_n = \frac{-2\lambda}{((\Phi_0^n \lambda - 1)^4 - 2)} a_{n-1}, \tag{6.142}
\]
must be satisfied. To check the resulting series converge the ratio test may be implemented. It can be seen that the ratio of two successive terms is given by
\[
 L = \lim_{n \to \infty} \left| \frac{a_n \tilde{\Phi}_0^n}{a_{n-1} \tilde{\Phi}_0^{n-1}} \right|,
\]
\[
= \left| \frac{-2\lambda}{((\Phi_0^n \lambda - 1)^4 - 2)} \right|,
\]
\[
= \lim_{n \to \infty} \left| \frac{-2\lambda}{((\Phi_0^n \lambda - 1)^4 - 2)} \right|,
\]
\[
= 0. \tag{6.143}
\]
Since \( L < 1 \) the series converges absolutely for all \( \tilde{t} \). Thus, for small \( \tilde{t} \), \( N_M(\tilde{t}) \) may be written as

\[
N_M(\tilde{t}) = Q(\tilde{t}) + \mathcal{O}(\tilde{t}^2),
\]

where

\[
Q(\tilde{t}) = \sum_{j=1}^{4} a_{j0} \tilde{r}_j \left\{ 1 - \frac{2\lambda}{(r_j \lambda + \lambda - 1)^4 - 2\tilde{t}} \right. \\
+ \left. \frac{4\lambda^2}{(r_j \lambda + 2\lambda - 1)^4 - 2} \right\},
\]

where the \( j \) correspond to the four different values of \( r \). It should be noted that the constants \( c_j \) have now been incorporated into the values of \( a_j \). Note, this approximation is not strictly first order but is of the same form as that used for the cytotoxic case described in Section 6.2.2.

Proceeding as with the cytotoxic case it is possible to compare the approximation given by Equation (6.145) with the analytic solution to Equation (6.130). In order to do this the parameters \( D_I \) and \( \lambda \) are chosen to have the arbitrary values of one and two respectively. The initial conditions given by Equation (6.129) are transformed into the Cauchy initial conditions in terms of the transformed variable \( \tilde{t} \) as

\[
N_M(0) = 1,
\]

\[
\left. \frac{dN_M(\tilde{t})}{d\tilde{t}} \right|_{\tilde{t} = \frac{1}{2}} = 0,
\]

\[
\left. \frac{d^2N_M(\tilde{t})}{d\tilde{t}^2} \right|_{\tilde{t} = \frac{1}{2}} = 0,
\]

and

\[
\left. \frac{d^3N_M(\tilde{t})}{d\tilde{t}^3} \right|_{\tilde{t} = \frac{1}{2}} = -0.
\]
Applying these initial conditions to Equation (6.145) gives

\[ a_{10} = -0.1966 + 0.1999i, \]

\[ a_{20} = 0.6074, \]

\[ a_{30} = -0.6140, \]

\[ a_{40} = -0.1966 - 0.1999i, \]

where \( a_{j0}, j \in \{1, 2, 3, 4\} \), correspond to \( r_j \) where

\[ r_j = \frac{1 + 2^{4j}}{2}. \] (6.148)

This approximation, together with the analytic solution to Equation (6.130) are plotted in Figure 6.8.

![Plot showing a first order approximation together with actual solution for the fourth order ODE given in Equation (6.130) with \( D_I = 1 \) and \( \lambda = 2 \).](image)

**Figure 6.8:** Plot showing a first order approximation together with actual solution for the fourth order ODE given in Equation (6.130) with \( D_I = 1 \) and \( \lambda = 2 \).

As with the cytotoxic case Figure 6.8 demonstrates that this technique for approximating the solution is in good agreement with the analytical solution. But further work is necessary to gain information about the behaviour of the whole population of cells. By using
the approximation for the population of cells in the $M$ compartment, given by Equation (6.145) and Equations (6.126) it is possible to obtain approximations for the populations of cells in the other three phases. A plot for the total cell count, $N_T(t)$, for the parameters $D_I = 1$ and $\lambda = 2$ is shown in Figure 6.9. As can be seen from Figure 6.9 for

![Total Normalised Cell Count $N_T(t)$](image)

Figure 6.9: Plot Showing an Approximation to the Total Cell Population with Cytostatic Effect with $D_I = 1$ and $\lambda = 2$.

the parameter values chosen the total number of cells does decline initially. To determine when this is the case, and when the minimum will occur it is necessary to consider the equations governing $N_T(t)$. By combining Equations (6.126) it is possible to obtain

$$
\frac{dN_T(t)}{dt} = (1 - 2D_I e^{-\lambda t}) N_M(t).
$$

(6.149)

If the total population is to decrease then $\frac{dN_T(t)}{dt}$ must be negative for some $t$. The drug concentration, and hence its effects on the population, are monotonically decreasing therefore if $\frac{dN_T(t)}{dt} < 0$ for some $t \geq 0$ then

$$
\left. \frac{dN_T(t)}{dt} \right|_{t=0} < 0,
$$

(6.150)
therefore

\[(1 - 2D_I) N_M(0) < 0. \quad (6.151)\]

From the initial conditions given in Equation (6.129) if Equation (6.151) is to be satisfied then

\[D_I > \frac{1}{2}. \quad (6.152)\]

Furthermore, if the population is initially in decline it is possible to find the time at which a minimum number of cells is present by finding when the derivative of \(N_T(t)\) is zero, i.e.

\[(1 - 2D_I e^{-\lambda t}) N_M(t) = 0. \quad (6.153)\]

It is clear that this will be a minimum by considering the physical nature of the system. Any effects of the drug will diminish resulting in the population being able to recover. If \(N_M(t) \neq 0\) then for Equation (6.153) to hold

\[t = \frac{\ln (2D_I)}{\lambda}. \quad (6.154)\]

Thus, when \(D_I = 1\) and \(\lambda = 2\) a minimum value of \(N_T(t) = 3.838\) will occur when \(t = \frac{\ln(2)}{2} \approx 0.347\), which is in good agreement with Figure 6.9. Unlike the cytotoxic case it is now clear that the time at which the minimum occurs is logarithmically proportional to the initial dose and inversely proportional to \(\lambda\). Intuitively this is correct since the drug decay is exponential and if the relative wash out rate is lower then the time taken for the minimum to be achieved will be greater. Furthermore, by substituting Equation (6.154) into the expression for \(N_T(t)\) it can be shown that as \(D_I\) increases the value of the minimum decreases.

### 6.3.3 Case \(\Pi : \epsilon = \mathcal{O}(1)\)

This case will not be considered further for the reasons discussed for Case II of the cytotoxic model.
6.3.4 Case III: $\epsilon \to \infty$

This case is similar to the third case for the cytotoxic model. The rescaling $\tilde{t} = \epsilon t = \frac{t}{\epsilon}$ is applied to Equations (6.126) and (6.128) giving

\[
\frac{1}{\epsilon} \frac{dN_{G_1}(\tilde{t})}{dt} = 2(1 - D(\tilde{t}))N_M(\tilde{t}) - N_{G_1}(\tilde{t}),
\]

\[
\frac{1}{\epsilon} \frac{dN_S(\tilde{t})}{dt} = N_{G_1}(\tilde{t}) - N_S(\tilde{t}),
\]

\[
\frac{1}{\epsilon} \frac{dN_{G_2}(\tilde{t})}{dt} = N_S(\tilde{t}) - N_{G_2}(\tilde{t}),
\]

\[
\frac{1}{\epsilon} \frac{dN_{M}(\tilde{t})}{dt} = N_{G_2}(\tilde{t}) - N_M(\tilde{t}),
\]

and

\[
\frac{dD(\tilde{t})}{dt} = -\lambda \tilde{\epsilon} D(\tilde{t}) - N_T(\tilde{t})D(\tilde{t}).
\]

Note the initial conditions remain unaffected by these rescalings, so

\[
N_i(\tilde{t}) = N_i(0) = 1\text{ where } i \in \{G_1, S, G_2, M\}.
\]

For the zeroth order approximation all $\tilde{\epsilon}$ terms may be ignored. Thus Equations (6.110) simplify to

\[
\frac{dN_{G_1}(\tilde{t})}{dt} = 0,
\]

\[
\frac{dN_S(\tilde{t})}{dt} = 0,
\]

\[
\frac{dN_{G_2}(\tilde{t})}{dt} (t) = 0,
\]

\[
\frac{dN_{M}(\tilde{t})}{dt} (t) = 0.
\]

From Equation (6.157) it is readily seen that $N_T = 4$. Making this substitution into Equation (6.156) and neglecting the term multiplied by $\tilde{\epsilon}$ gives

\[
\frac{dD(\tilde{t})}{dt} = -4D(\tilde{t}).
\]
Hence,

\[ D(t) = D_I e^{-4t}. \]  

(6.160)

Equation (6.160) may now be expressed in terms of the original time variable, \( t \), as

\[ D(t) = D_I e^{-4t}. \]  

(6.161)

But \( \epsilon \to \infty \) therefore \( D(t) \approx 0 \). Thus, for the case \( \epsilon \to \infty \) the drug has a negligible uptake and has a minimal effect on the population. This means the population of cells grows in the same manner as the untreated model described in Section 2.1.2.

### 6.4 Comparison of the Effects of Cytotoxic and Cytostatic Drug Interactions

For both the cytotoxic and cytostatic models Case II is disregarded and only Case I and Case III are considered. For both types of drug interaction Case III results in the drug having negligible effect on the population resulting in normal growth. This only leaves Case I for comparison.

The \( G_1 \) phase in the cytotoxic model and the \( M \) phase in the cytostatic model are governed by two fourth order ODEs, given by Equations (6.26) and (6.130) respectively. These different fourth order ODEs appear to be of a similar form. Upon obtaining the approximations it is clear they do share common features, this is apparent due to the \( r \) values that were obtained in the construction of the approximations being the same, i.e. \( r = \frac{1+2\lambda}{\lambda} \), \( \frac{1+2\lambda}{\lambda} \). By comparing the approximate solutions to Equations (6.26) and (6.130) it can be seen that as \( t \to \infty \) then both solutions tend to \( c \left( e^{-2(t-\delta)} \right)^{-2\frac{1}{2}} \) where \( c \) is a constant, this is illustrated in Figure 6.10. Therefore, if the long term behaviour of a cytotoxic model is given by \( a \left( e^{-2(t-\delta)} \right)^{1-2\frac{1}{2}} \) then this may be expressed as \( b \left( e^{-2(t-\delta-\rho)} \right)^{1-2\frac{1}{2}} \) where the constants \( a \) and \( b \) are related via \( b = a \left( e^{2\rho} \right)^{1-2\frac{1}{2}} \). Hence it is possible to view the cytotoxic model as the cytostatic model with a time delay of \( \rho \). The idea that both models only differ by a time shift is reinforced by comparing the original systems of four first order ODEs governing the models.

The ability to use either model may be further justified by considering how the different models effect the population over a period of time. In both models a proportion of cells
is stopped from dividing, either by killing them directly (cytotoxicity) or making division impossible (cytostasis). For the population it is irrelevant how these cells have been made non viable, it is only important that they are unable to contribute to the next generation of cells. It therefore makes sense that either model may be used. For the examples given in Sections 6.2 and 6.3 the population distributions are equal at $t = 0$ it therefore seems biologically realistic that the cytostatic drug would have a greater effect initially as this immediately effects how many cells are able to divide.

### 6.5 ODE Drug Interaction Summary

In this chapter a generic, four compartment, ODE cell cycle model was constructed which accounts for multiple ways in which drug interactions may effect a population of cells. Since only a qualitative understanding of the system is sought some assumptions were made which in turn simplified the system of equations governing the model.
In Section 6.2 the model was refined to take into account the effects of a cytotoxic drug on the population of cells. The modified system of equations were presented and non-dimensionalised. It was observed that the model may be divided into three categories dependent on the order of one of the parameters, $c_i$, of the system. It was discussed that one of these cases could be removed as it only applied to a very limited range of biological parameters, leaving only two cases to be considered. One of these was shown to have a trivial solution, leaving only one of interest. For Case I in the cytotoxic model an analytic solution in terms of hypergeometric functions was found. However, due to the model being simplified, it may not be possible to find an analytical solution for a system which has had fewer simplifications. As such, a technique for obtaining an approximate solution was given. This technique was first demonstrated on a simpler system which has similarities to the biological system of interest. Although this technique was only applied to the special case where all $k_i$ are equal it may be extended to apply to any system where the $r$ values obtained in solving the equation for the $a_0$ coefficient are linearly independent. This enabled the approximation to easily be compared with the analytical solution. Once the technique had been verified on the simpler system it was used on the cytotoxic model and the results compared once more to the analytical solution. Once the approximation was verified against the analytic solution for a single phase an approximation was obtained for the total cell population. It was also shown that from Equations (6.16) it was possible to derive a single ODE for the total population of cells, Equation (6.105). From this equation it was shown that for $D_I > 1$ there is an initial decline in the overall cell population and the time taken for the overall population to attain its minimum value was determined.

Section 6.3 followed a similar format to Section 6.2 with the model now being refined to take into account the effects of a cytostatic drug on the population of cells. As this scenario was worked through a number of similarities between the cytotoxic and cytostatic model were noted.

In Section 6.4 the cytotoxic and cytostatic models were compared. It was shown that for long term qualitative predictions both models produce the same results. The rationale for why this may be the case was then discussed. It was therefore concluded that either model may be used for modelling the effects of a simple drug interaction with a population of cells.
Chapter 7

Conclusion and Further Work

In this thesis the modelling of two biological processes, namely the cell cycle and PKPD interaction have been considered. In Chapters 2, 3 and 4 different approaches for modelling a population of cells were discussed. In Chapter 5 some common techniques for modelling PKPD were introduced. In Chapter 6 these two different threads were brought together and a framework for modelling a population of cells with drug interaction was presented.

7.1 Summary

7.1.1 Summary of Chapter 2 : Cell Cycle Models

In Chapter 2 a number of existing cell cycle models were considered. Some of the approaches utilising ODEs to model a population of cells were discussed in Section 2.1 and it was shown that using Steel’s formulae, which were derived in Section 2.1.1, it is possible to obtain further information from a single unstructured ODE model. Structured and molecular ODE models were also discussed.

Since an ODE based model does not consider the intraphase heterogeneity of the population PDE models are often used. Most cell population models that utilise PDEs have a population balance framework. A comprehensive review of existing population balance models was given in Section 2.2.1. The review starts with a brief introduction of the population balance framework, together with an overview of the most common types of structuring used for a population of cells. The concept of partition functions for mass and DNA structured population balance models was introduced and the advantage of not
needing a partition function for an age structured model was also discussed. DNA, mass
and multi variable models were reviewed with both a single, generation type approach and
a multi-cell phase approach being considered. A detailed review of the DNA structured
framework presented by Basse et al. in [3] was undertaken and a number of potential
problems with this model were discussed.

In Section 2.2.1 age structured PDEs are considered. The structure used in [6] was
reviewed and whilst this work provides a good general framework for the solution of a multi
compartment, age structured model the assumption that the transition of cells between
the $S$ and $G_1$ phases in this model is independent of age, is biologically unrealistic. These
issues are addressed by the CelCyMUS model given by [22], [23] and [57]. In Section 2.2.1
the age structured PDE model, CelCyMUS, was reviewed in depth. This work consists
of a detailed and accurate model, which was verified against experimental data, and has
since been extended to consider external factors such as radiation [35]. Despite this model
providing a good fit to experimental data there are issues concerning the validity of the
transition functions used in [23] and [57]. A number of simplifications may be made
without compromising the model’s ability to accurately simulate and fit experimental
data.

7.1.2 Summary of Chapter 3 : Validation and Comparison of New Ordinary
Differential Equation and Partial Differential Equation Models

In Chapter 3 two new models were formulated. The first of these was a structured ODE
model consisting of four compartments, each corresponding to a different phase of the
cell cycle. General results for the phase distribution of the population were derived. The
phase distributions obtained using this model were compared to Steel’s formulae, and
it was shown in the special case when all the rate constants are unity the results are
identical. For the case when the rate constants are not all unity estimates were placed
on the upper bound for the difference between Steel’s formulae and this ODE modelling
approach.

Using the CelCyMUS model as a starting point a new three compartment, age-structured
PDE model was presented in Section 3.2. Section 3.3 contains a comprehensive review of
the transition functions which model how the cells progress from the $G_1$ to $S$ phase of the
cycle. Existing constant [6], quadratic [22] and [23], and sigmoidal [57] transition functions
were discussed in detail. Motivated, by the sigmoidal transition function given in [57] a new sigmoidal transition function was presented. When expressed in non-dimensionalised form this new sigmoidal transition function was shown to have only one independent parameter, thus making the problem more tractable.

In Section 3.4 a numerical scheme for the new age-structured model was presented, together with a discussion on the scheme’s stability. It was shown that for a monotonically increasing probability of transition the numerical scheme is stable.

Section 3.5 saw the new age structured model with both constant and sigmoidal transition functions compared with experimental data. It was shown that a constant transition rule is sufficient to accurately fit the experimental growth curve. The blocking process used in the experiment considered introduces oscillations into the phase distribution of the cells which could have exacerbated the measuring of the cell population, leading to a large experimental error. Because of this, if information concerning the phase distribution for the cell population is important then the form of the transition function becomes more critical.

The ODE model framework discussed in Section 3.1 was modified to allow direct comparison with the PDE model described in Section 3.2. All of the parameter values obtained in the ODE optimisation provided a good match to the experimental cell growth curve. The fraction of cells in the different compartments varies by 19% and whilst lying outside the bounds of experimental error ([18], [19] and [36]) this is still sufficiently accurate to gain a qualitative understanding of the behaviour of the cell population.

From this comparison it was concluded that if the duration of the phases is not important and the only information required is a qualitative understanding of the phase distribution or an approximation on the total number of cells within the population then a simple ODE model may be used. If a more accurate measure of the phase distribution of the cell population is required then a PDE or integral equation model may be required.

7.1.3 Summary of Chapter 4: Integral Equations for an Age Structured Population Balance Model

In Chapter 4 the construction of integral equations which may be used for the analysis of the cell population was considered. Two new functions, $M(t)$ and $P(t)$, representing the
number of cells dividing and the total number of cells in the population were introduced. The system was then expressed in terms of integral equations for these two new functions. Once \( M(t) \) and \( P(t) \) had been found the integral equations for the population densities were readily derived. No analysis of these integral equations was undertaken and they were included in this thesis to illustrate the availability of different techniques for modelling a population of cells using a population balance framework. Numerical calculations involving the integral equations may provide a faster alternative to using a finite difference scheme for solving the PDEs directly but this has not been investigated further.

7.1.4 Summary of Chapter 5: Pharmacokinetic and Pharmacodynamic Models

Chapter 5 started by giving a brief overview of how PKPD may be modelled. In the case of pharmacodynamics the way in which this may be incorporated directly into a cell cycle model was discussed. A brief introduction to widely used pharmacokinetic modelling was then undertaken. Drugs administered by intravenous infusion and also extravascularly were considered and simple compartment models described. The idea of multiple periodic doses resulting in an iterative map of ODEs was discussed, but not solved. It was explained how the effects of bioavailability may be included and what effect this would have on the resulting solution.

It was shown that as a first approximation to gain a qualitative understanding of how a system behaves it is possible to use a single compartment model. Furthermore, it was shown, in Section 5.1.1, that it was possible to back extrapolate from the graph of the concentration caused by a constant rate infusion to obtain the equivalent bolus dose which would give the concentration curve post infusion. As such to simplify the model further the dose may be assumed to be administered in a single bolus dose.

7.1.5 Summary of Chapter 6: A Cell Cycle Model with Drug Interaction

In Chapter 6 a generic, four compartment, ODE cell cycle model was constructed which accounts for multiple ways in which drug interactions may effect a population of cells. Since only a qualitative understanding of the system was sought some assumptions were made which in turn simplified the system of equations governing the model.
Section 6.2 the model was refined to take into account the effects of a cytotoxic drug on the population of cells. The modified system of equations were presented and non-dimensionalised. It was observed that the model may be divided into three categories dependent on the order of one of the parameters, $\epsilon$, of the system. It was discussed that one of these cases could be removed as it only applied to a very limited range of biological parameters, leaving only two cases to be considered. One of these was shown to have a trivial solution, leaving only one of interest. For this remaining case an analytic solution in terms of hypergeometric functions was found. However, due to the model being simplified, it was noted it may not be possible to find an analytical solution for a system which has had fewer simplifications. As such, a technique for obtaining an approximate solution was given. This technique was first demonstrated on a simpler system. Although this technique was only applied to the special case where all $k_i$ are equal it may be extended to apply to any system where the $r$ values obtained in solving the equation for the $a_0$ coefficient are linearly independent. This enabled the approximation to easily be compared with the analytical solution. Once the technique had been verified on the simpler system it was used on the cytotoxic model and the results compared once more to the analytical solution. An approximation was then obtained for the total cell population $N_T$ and it was shown that it was possible to determine if the population was monotonically increasing or if there was an initial dip in the population size due to the drug interaction, and if so when the minimum number of cells would occur. Such information is of importance in calculating when multiple doses should be given to optimise any effects.

Section 6.3 followed a similar format to Section 6.2 with the model now being refined to take into account the effects of a cytostatic drug on the population of cells. As this scenario was worked through a number of similarities between the cytotoxic and cytostatic model were noted.

In Section 6.4 the cytotoxic and cytostatic models were compared. It was shown that for long term qualitative predictions both models tended to the same limit, differing only by a time delay. The rationale for why this may be the case was then discussed. It was therefore concluded that either model may be used for modelling the effects of a simple drug interaction with a population of cells.
7.2 Conclusion

Whilst PDE models are necessary to capture the intraphase heterogeneity of a population of cells if the only information required is a qualitative understanding of the phase distribution or an approximation on the total number of cells within the population then a simple ODE model may be used. For a qualitative understanding of a PKPD system it was shown it is possible to use a single compartment model with a single bolus dose included in the initial conditions. Combining such models for the cell population and the PKPD effects it is possible to obtain a system of equations which can be solved to give a semi analytical solution. Whilst the models presented in Chapter 6 are too simplistic to be used to predict experimental results it is hoped this work provides a solid basis upon which such models may be developed further.

7.3 Further Work

In this section several ideas for extending this work are discussed.

7.3.1 Overcrowding

The cell cycle models discussed in this thesis do not take into account the possible effects of the physical overcrowding of the cells. Since there is no spatial element included in the models, once any transient effects have diminished the population will grow exponentially. In reality the physical limitations on space mean the cell population cannot remain in an exponential growth phase. To stop the exponential phase of cell growth continuing indefinitely it is necessary to introduce some form of overcrowding control. There are three possible ways the population size may be regulated.

For the age-structured PDE model discussed in Section 3.2 it is possible to control the probability of cells passing through the $G_1 - S$ checkpoint via the transition function. If the probability of transition is dependent on nutrient uptake and there are limited nutrients then this would clearly reduce the number of cells progressing through the cycle.

A transition function for limited nutrients is briefly discussed in Appendix A. If nutrients are not a limiting factor for the growth of the cell population then it is a reasonable assumption to keep the uptake rate, $R$ constant. If the ODE model discussed in Section 3.1 is used similar changes could be made to one of the rate constants $k$. 
The second method is to introduce some form of survival fraction which is dependent on total population size. In effect this introduces a loss term into the differential equations for the cell population densities. Such a method has been considered in [17] and [28].

An alternative approach is to vary the proliferation rate at cell division. Clearly, a cell either divides or it does not but it is possible to attribute an average number of daughter cells produced for the population. If such a division rate is dependent on population size this would have the effect of regulating the population’s total size. This concept has been explored by [17] and [28].

It is the latter of these approaches which is now considered further, with a new birth rate function being introduced. Consider an artificial cell line for which if the cells do not have many neighbours (i.e. very low cell density) then the population dies, so for low cell density the proliferation factor needs to be less than one. For moderate cell densities then the growth is optimum and therefore the proliferation factor needs to be close to two. For high cell densities the cells will be overcrowded therefore will have a lower proliferation factor. If when overcrowded the proliferation factor is greater than one then the population may continue to increase (depending on how many cells die due to other events in the cycle) making the overcrowding worse. It therefore seems a reasonable assumption to make the proliferation factor at high cell densities to be slightly less than one. A suitable type of function which fulfills these criteria is given by

\[ 1 - c + \tanh(N(t) - a) - c \tanh(N(t) - b), \]  \hspace{1cm} (7.1)

where \( N(t) \) is the total cell density and \( a, b \) and \( c \) are parameters which determine the shape and asymptotic limit of the function as \( N(t) \) increases. A sketch of this function is shown in Figure 7.1. This function asymptotes to \( 2 - 2c \) as \( N(t) \to \infty \) and has a maximum value of two which approximately occurs when \( N(t) \in [a, b] \). If the cell density is normalised so the starting population has a density of one then values of 0.1, 10 and 0.625 for \( a, b \) and \( c \) respectively give a function which has a maximum of two for cell densities varying approximately between 0.1 and 10 times the starting density. The asymptote is now 0.75 which also satisfies the requirement that the asymptote is less than one. It is clear that the introduction of such a function to regulate the proliferation factor will result in the population reaching a locally stable steady state at a normalised population of 10.
Figure 7.1: Sketch of A Suitable Overcrowding Function for which Cell Growth is Optimal at Moderate Cell Densities.

To demonstrate the effect of introducing this overcrowding term, the new age structured model described in Section 3.2 with the new sigmoidal transition function was run using the parameters given in Table 3.1 together with the overcrowding term using the parameters given above. The resulting growth curve is shown in Figure 7.2. This plot only displays viable cells, as such it starts at zero since cells entering the cycle from the $G^0$ are not included.

By comparing Figures 7.2 and 3.12b it can be seen that the transient oscillations discussed in Section 3.5.1 decay at a faster rate. This is possibly due to the stiff nature of the function given in Equation (7.1). Different approaches for taking into account the effects of overcrowding, such as competition between cells and also competition with other cellular populations has not been considered. This may provide more biologically meaningful results.
Figure 7.2: The Effect of Introducing an Overcrowding Function on the Cell Density.

7.3.2 Periodic Dosing

The idea of periodic dosing was discussed in Section 5.1.3. It was shown that it is possible to express the drug concentration as a set of iterative differential equations where the initial conditions for the \( n^{th} \) period of infusion/post infusion is the final concentration in the \( (n - 1)^{th} \) period. By combining this scheme with a cell cycle model it should be possible to accurately simulate the effects of periodic dosing. However, it should be noted that for periodic dosing it is possible that a scenario corresponding to Case II in Sections 6.2 and 6.3 (i.e. \( \mathcal{O}(\epsilon) = 1 \)) may no longer only apply for a small parameter space and as such this case will need to be considered.

A good starting point for the time spacing between the periodic doses would be the time taken for any minimum in the total population to occur. However, this may not be the optimal strategy as this does not take into consideration the phase distribution of the cells. It may therefore prove useful to consider when minimum cell densities occur within a particular phase or when maximum relative phase density occurs for multiple dosing.
7.3 Further Work

7.3.3 Multiple Compartments

Using a population balance framework, heterogeneous populations of cells may be modelled mathematically, resulting in a set of partial differential equations for the cell population density. If the heterogeneity is not of interest then ordinary differential equation models suffice. In both cases, the models may then be extended to account for external factors affecting the population such as nutrient deprivation, radiation treatment or cytotoxic drugs allowing for a greater understanding of the carcinogenesis process. The model may be refined still further by considering sub populations within the tumour which are grouped according to a particular attribute, such as spatial location or proximity to a nutrient source, i.e. how close cells are to the vascular network.

Tumour Vascular Networks

Malignant tumours often experience rapid growth resulting in regions of the tumour becoming hypoxic. Cells in these hypoxic regions are normally more resistant to treatment [29] and [51]. These regions of hypoxia promote the angiogenesis process within the tumour resulting in avascular growth. The random nature of tumour vascular networks means there are often regions within the same tumour which are well perfused, hypoxic and necrotic. Further details about the oxygenation of tumours may be found in [56].

Outline of a Multiple Compartment Model

By considering these populations separately it is possible to build a multiple compartment model with each compartment representing a different level of oxygenation. Whilst this approach lacks a spatial element it does allow a qualitative picture of the behaviour and growth of cells in each compartment to be obtained. As discussed in Chapter 5 the drug is delivered to the target cells via the blood plasma. Therefore, it is a reasonable assumption that the amount of drug received at a particular site is proportional to the level of oxygenation. It should be noted however, that the distance a drug diffuses through the inter cellular media is different to the distance oxygen diffuses [34], but since the model outlined here accounts for ranges of hypoxia / oxygen proliferation this is accounted for via different rate constants.

A suitable three compartment model is shown in figure 7.3.
Figure 7.3: A Combined PKPD and Cell Cycle Three Compartment Model.
The full set of equations for this model are now given by

**Well Perfused Region**

Cell Population density equations

\[
\frac{dN_{G_1}(t)}{dt} = P_n(D_n(t))k_1(D_n(t))N_M(t) - k_2(D_n(t))N_{G_1}(t) - f_1(D_n(t))N_{G_1}(t), \quad (7.2a)
\]

\[
\frac{dN_S(t)}{dt} = k_2(D_n(t))N_{G_1}(t) - k_3(D_n(t))N_S(t) - f_2(D_n(t))N_S(t), \quad (7.2b)
\]

\[
\frac{dN_{G_2}(t)}{dt} = k_3(D_n(t))N_S(t) - k_4(D_n(t))N_{G_2}(t) - f_3(D_n(t))N_{G_2}(t), \quad (7.2c)
\]

\[
\frac{dN_M(t)}{dt} = k_4(D_n(t))N_{G_2}(t) - k_1(D_n(t))N_M(t) - f_4(D_n(t))N_M(t). \quad (7.2d)
\]

Drug concentration equation

\[
\frac{dD_n(t)}{dt} = d_1(t) + d_8(t)D_h(t) - d_7(t)D_n(t) - d_4(t)D_n(t) - g_n(N_{G_1}(t), N_S(t), N_{G_2}(t), N_M(t))D_n(t). \quad (7.3)
\]

**Hypoxic Region**

Cell Population density equations

\[
\frac{dH_{G_1}(t)}{dt} = P_h(D_h(t))k_5(D_h(t))H_M(t) - k_6(D_h(t))H_{G_1}(t) - f_5(D_h(t))H_{G_1}(t), \quad (7.4a)
\]

\[
\frac{dH_S(t)}{dt} = k_6(D_h(t))H_{G_1}(t) - k_7(D_h(t))H_S(t) - f_6(D_h(t))H_S(t), \quad (7.4b)
\]

\[
\frac{dH_{G_2}(t)}{dt} = k_7(D_h(t))H_S(t) - k_8(D_h(t))H_{G_2}(t) - f_7(D_h(t))H_{G_2}(t), \quad (7.4c)
\]

\[
\frac{dH_M(t)}{dt} = k_8(D_h(t))H_{G_2}(t) - k_5(D_h(t))H_M(t) - f_8(D_h(t))H_M(t). \quad (7.4d)
\]

Drug concentration equation

\[
\frac{dD_h(t)}{dt} = d_2(t) + d_7(t)D_n(t) - d_8(t)D_h(t) + d_{10}(t)D_c(t) - d_9(t)D_h(t) - d_5(t)D_h(t) - g_h(H_{G_1}(t), H_S(t), H_{G_2}(t), H_M(t))D_h(t). \quad (7.5)
\]
Necrotic Region

Cell Population density equations

\[ \frac{dC_{G1}(t)}{dt} = P_c(D_c(t))k_9(D_c(t))C_M(t) - k_{10}(D_c(t))C_{G1}(t) - f_9(D_c(t))C_{G1}(t), \]  
\[ \frac{dC_S(t)}{dt} = k_{10}(D_c(t))C_{G1}(t) - k_{11}(D_c(t))C_S(t) - f_{10}(D_c(t))C_S(t), \]
\[ \frac{dC_{G2}(t)}{dt} = k_{11}(D_c(t))C_S(t) - k_{12}(D_c(t))C_{G2}(t) - f_{11}(D_c(t))C_{G2}(t), \]
\[ \frac{dC_M(t)}{dt} = k_{12}(D_c(t))C_{G2}(t) - k_9(D_c(t))C_M(t) - f_{12}(D_c(t))C_M(t). \]

Drug concentration equation

\[ \frac{dD_c(t)}{dt} = d_3(t) + d_9(t)D_h(t) - d_{10}(t)D_c(t) - d_6(t)D_c(t) \]
\[ - g_c(C_{G1}(t), C_S(t), C_{G2}(t), C_M(t))D_c(t). \]

Equations (7.2a)-(7.7), together with suitable boundary conditions fully define the system.

Note, this model does not account for cells passing between the different compartments. The reason for this not being included is the angiogenesis process and cell motility are likely to occur on a timescale much greater than that being considered for drug interactions.

7.3.4 Competition

The population of healthy cells near the tumour may be included in the model outlined in Section 7.3.3 by the addition of an additonal compartment. This extra compartment would have the same rate constants as the well perfused region but additionally may include some form of competition between the healthy cells and the well perfused tumour cells as these would be competing for space and nutrients. One possible way in which this may be done is by adding an overcrowding term to the populations of healthy and well perfused tumour cells. Such an overcrowding term may take the form described in Section 7.3.1 where \( N(t) \) is now the sum of both of these populations.

Including the healthy cell population in any model for oncology treatments is important as this allows for the toxicity effects on the healthy population to be included.
Appendix A

Modified Sigmoidal Transition Function, Accounting for the Effects of Limited Nutrient Availability

To properly take into account the effects of limited nutrient availability on the cells we need to return to the assumptions made about the rate of nutrient uptake. When the availability of nutrients is limited an alternative to the constant uptake rate is required, a reasonable choice is to take a Michaelis-Menten type form such as

\[ R = \frac{R_{\text{max}} C_{\text{glut}}}{K_s + C_{\text{glut}}}, \quad (A.1) \]

where \( R_{\text{max}} \) is the maximum rate glutamine may be absorbed, \( C_{\text{glut}} \) is the remaining amount of glutamine in the medium and \( K_s \) is a parameter to be determined.

Note, \( R \) is now a function of \( C_{\text{glut}} \) which is itself a function of time, however for ease \( R(C_{\text{glut}}(t)) \) is simply written as \( R \) and \( C_{\text{glut}}(t) \) as \( C_{\text{glut}} \). Inserting this form for glutamine
uptake into the new sigmoidal transition rule presented in Section 3.3.1 yields

\[
h(\tau) = \frac{R \cdot C_c}{S} \exp \left( \frac{C_c - \theta}{\theta} \right),
\]

\[
= \frac{R \cdot \theta}{S} \exp \left( \frac{C_c - \theta}{\theta} \right),
\]

\[
= \frac{R_{\max} C_{\text{glut}}}{K_s + C_{\text{glut}}} \frac{\theta}{1 + \exp \left( -\frac{C_c - \theta}{\theta} \right)}.
\]  

(A.2)

The non-dimensionalisation of this form of the transition rule is the same as in Section 3.3.1, however the rescaling is set so the maximum amount of glutamine a cell can uptake in each unit of time is one. This was chosen to occur above the initial medium glutamine concentrations.
Appendix B

Construction of the Time Step Matrix

The matrix $M$ given in Equation (3.97) is composed of smaller sub matrices. Each of these sub matrices relate to part of the time stepping of cells in the $X$ and $Y$ phases. $M$ may be written as

\[
M = \begin{bmatrix}
0 & 0 & 0 & 0 & A \\
B & C & D & 0 & 0 \\
E & F & 0 & 0 & 0 \\
G & H & I & 0 & 0 \\
0 & 0 & J & K
\end{bmatrix}
\]  \hspace{1cm} (B.1)

The sub matrices $A$-$K$ are defined as follows

$A$ represents cells leaving the $Y$ phase, doubling and entering the $X$ phase. It is a $(K-1) \times 1$ matrix containing only one non-zero entry

\[
\begin{bmatrix}
0 & \cdots & 0 & 2
\end{bmatrix}
\]  \hspace{1cm} (B.2)

$B$, $C$ and $D$ form the main central difference scheme i.e. Lax-Wendroff. $B$ is an $1 \times (N-2)$
matrix whose first cell is the only non-zero entry

\[
\begin{bmatrix}
\times \\
0 \\
\vdots \\
0
\end{bmatrix},
\] (B.3)

\(C\) is an \((N-2)\times(N-2)\) tri-diagonal matrix

\[
\begin{bmatrix}
\times & \times & 0 & \ldots & \ldots & 0 \\
\times & \times & \times & \ddots & \vdots & \vdots \\
0 & \times & \times & \times & \ddots & 0 \\
\vdots & \ddots & \ddots & \ddots & \ddots & \ddots \\
\vdots & \ddots & \times & \times & \times & \times \\
0 & \ldots & \ldots & \times & \times & \times
\end{bmatrix},
\] (B.4)

and \(D\) is a \(1\times(N-2)\) matrix whose last cell is the only non-zero entry

\[
\begin{bmatrix}
0 \\
\vdots \\
0 \\
\times
\end{bmatrix},
\] (B.5)

\(E\) and \(F\) contain the finite difference scheme for the last age component of the X phase. This can either be a backward difference scheme or a central difference scheme with the \(X^{\tau+1}\) term assumed to be zero and therefore omitted. Using the central difference scheme (with \(X^{\tau+1}\) term omitted) \(E\) is a \(1\times(N-2)\) matrix whose last cell is the only non zero entry

\[
\begin{bmatrix}
0 & \ldots & 0 & \times
\end{bmatrix},
\] (B.6)

and \(F\) is a single non-zero entry matrix

\[
\begin{bmatrix}
\times
\end{bmatrix}.
\] (B.7)

\(G\), \(H\) and \(I\) represent the cells leaving the X phase and entering Y phase via the transition function \(h(v)\). The number of cells leaving X at any time step via the transition rule is given by

\[
\int_0^{X_{max}} h(\tau) X d\tau.
\] (B.8)
For discretization purposes this can be approximated using the trapezium rule
\[
Y_{i+1}^0 = \int_0^{N_{\text{max}}} h(\tau) X \, d\tau = \frac{\alpha}{2} (h(0) X_i^0 + h(N - 1) X_i^{N-1}) + \alpha \sum_{j=1}^{N-2} h(j) X_i^j, \tag{B.9}
\]
therefore \( G \) is a single celled matrix
\[
\begin{bmatrix}
\frac{\alpha}{2} h(0)
\end{bmatrix}, \tag{B.10}
\]
\( H \) is a \((N-2)\times1\) matrix given by
\[
\begin{bmatrix}
\alpha h(1) & \cdots & \alpha h(N - 2)
\end{bmatrix}, \tag{B.11}
\]
and \( I \) is a single celled matrix
\[
\begin{bmatrix}
\frac{\alpha}{2} h(N - 1)
\end{bmatrix}. \tag{B.12}
\]
\( J \) and \( K \) represent cells moving through \( Y \). \( J \) is a \( 1 \times (K-1) \) matrix containing a 1 in the first cell and zeros in all other cells.
\[
\begin{bmatrix}
1 \\
0 \\
\vdots \\
0
\end{bmatrix}, \tag{B.13}
\]
and \( K \) contains ones on the lower diagonal and zeros elsewhere
\[
\begin{bmatrix}
0 & 0 & \cdots & \cdots & 0 \\
1 & 0 & \cdots & \cdots & 0 \\
0 & \ddots & \ddots & \vdots & \\
0 & \ddots & \ddots & \ddots & 0 \\
0 & \cdots & 0 & 1 & 0
\end{bmatrix}. \tag{B.14}
\]
Appendix C

An Alternative Approach for the Proof of Stability of the Numerical Scheme

In Section 3.4 a numerical scheme for the simplified model is discussed. To prove the stability of the numerical scheme it is sufficient to show that a simpler numerical scheme, for a two compartment model is also stable. As detailed in Section 3.4 this scheme may be written as

\[
\begin{bmatrix}
X_{m+1}^0 \\
X_{m+1}^{1..N-2} \\
X_{m+1}^{N-1} \\
Y_{m+1}^0 \\
Y_{m+1}^{1..K-1}
\end{bmatrix}
= M
\begin{bmatrix}
X_m^0 \\
X_m^{1..N-2} \\
X_m^{N-1} \\
Y_m^0 \\
Y_m^{1..K-1}
\end{bmatrix},
\]

(C.1)

where \( M \) is an \((N + K) \times (N + K)\) matrix. To prove the stability of this scheme it is sufficient to show the norm of \( M \) satisfies

\[
\left\| M \right\| \leq 1 + \kappa \alpha,
\]

(C.2)

where \( \delta t = \delta \tau = \alpha \) and \( \kappa \) is a constant independent of \( \alpha \). An argument following that described on p66-67 of [58] shows that such a \( \kappa \) can be found. Note that this \( \kappa \) depends on a bound for \( \left\| M \right\| \) where \( j \leq N \), this is allowed by the theory discussed in [58]. The norm of \( M \) is given by

\[
\left\| M \right\| = \sup\{2, \alpha \sum_{j=1}^{N-2} h(j) + \frac{\alpha}{2} (h(0) + h(N - 2))\}.
\]

(C.3)
By definition $\alpha = \frac{X_{\text{max}}}{N}$, also since $h(v)$ is the probability of transition $\left| h(i) \right| \leq 1 \forall i$ therefore
\[
\alpha \sum_{j=1}^{N-2} h(j) = \frac{X_{\text{max}}}{N} \sum_{j=1}^{N-2} h(j) \leq \frac{X_{\text{max}}(N - 2)}{N},
\]
and therefore remains bounded. Therefore $\| M \| \leq 1 + \kappa \alpha$ so the scheme is stable.
Appendix D

Change of Order of Integration for $P_C(t)$

In order to change the order of integration on the second of the integrals in Equation (4.55) it is helpful to consider a graphical representation of the region of interest.

Figure D.1: Graphical Representation of the Region of Integration for $P_C(t)$ when $T_C \leq t < T_B$.

From considering this graphical representation of the region of integration it can be seen that

$$
\int_0^{T_C} \int_0^{t-\tau} d\sigma \, d\tau = \int_0^{t-T_C} \int_0^{T_C} d\tau \, d\sigma + \int_{T_C}^t \int_0^{t-\sigma} d\tau \, d\sigma.
$$

(D.1)
Appendix E

A Check of the Numerical Approximations for the Solutions to ODEs

E.1 Second Order ODE Solution Comparison

The analytic solution to Equation (6.30) given by Equation (6.31) may be expressed in terms of $\tilde{t}$, as given in Equation (6.33), as

$$x(t) = \tilde{A}e^{\tilde{t}^\frac{\lambda+2}{2\lambda}} \left( I_{\frac{\lambda}{2\lambda}}(\tilde{t}) + i \frac{\lambda}{2\lambda} (\tilde{t}) \right)$$

$$+ \tilde{B}e^{\tilde{t}^\frac{\lambda+2}{2\lambda}} \left( K_{\frac{\lambda+2}{2\lambda}}(\tilde{t}) - K_{\frac{\lambda+2}{2\lambda}}(\tilde{t}) \right),$$

(E.1)

where $\tilde{A}$ and $\tilde{B}$ are new constants. Upon making the substitution $\lambda = 5$ Equation (E.1) becomes

$$x(t) = \tilde{A}e^{\tilde{t}^\frac{7}{30}} \left( I_{\frac{7}{30}}(\tilde{t}) + i \frac{7}{30} (\tilde{t}) \right)$$

$$+ \tilde{B}e^{\tilde{t}^\frac{7}{30}} \left( K_{\frac{7}{30}}(\tilde{t}) - K_{\frac{7}{30}}(\tilde{t}) \right).$$

(E.2)

$I_{\alpha}$ and $K_{\alpha}$ may be expressed as

$$I_{\alpha} = \sum_{r=0}^{\infty} \frac{(\frac{1}{2})^{\alpha+2r}}{r!\Gamma(\alpha + r + 1)},$$

(E.3)

and

$$K_{\alpha} = \frac{\pi}{2} \left( I_{-\alpha}(\tilde{t}) - I_{\alpha}(\tilde{t}) \right) \cosec(\alpha \pi),$$

(E.4)

respectively.
Therefore,
\[ I_{\frac{-3}{10}}(\bar{t}) = \frac{2^{\frac{3}{10}} 2^{\bar{\pi}^3}}{\Gamma\left(\frac{7}{10}\right)} + \frac{2^{\frac{17}{10}} 2^{\bar{\pi}^7}}{\Gamma\left(\frac{17}{10}\right)} + O\left(\bar{t}^{\frac{27}{10}}\right), \quad (E.5) \]
\[ I_{\frac{7}{10}}(\bar{t}) = \frac{2^{\frac{7}{10}} 2^{\bar{\pi}^7}}{\Gamma\left(\frac{17}{10}\right)} + O\left(\bar{t}^{\frac{27}{10}}\right), \quad (E.6) \]
\[ K_{\frac{-3}{10}}(\bar{t}) = \frac{\pi}{2} \left( I_{\frac{-3}{10}}(\bar{t}) - I_{\frac{7}{10}}(\bar{t}) \right) \csc\left(\frac{3\pi}{10}\right), \]
\[ = \frac{\pi}{2} \left( \frac{2^{\frac{3}{10}} 2^{\bar{\pi}^3}}{\Gamma\left(\frac{7}{10}\right)} + \frac{2^{\frac{17}{10}} 2^{\bar{\pi}^7}}{\Gamma\left(\frac{17}{10}\right)} - \frac{2^{\frac{3}{10}} 2^{\bar{\pi}^3}}{\Gamma\left(\frac{17}{10}\right)} \right) \csc\left(\frac{3\pi}{10}\right) + O\left(\bar{t}^{\frac{27}{10}}\right), \quad (E.7) \]
and
\[ K_{\frac{7}{10}}(\bar{t}) = \frac{\pi}{2} \left( I_{\frac{-3}{10}}(\bar{t}) - I_{\frac{7}{10}}(\bar{t}) \right) \csc\left(\frac{7\pi}{10}\right), \]
\[ = \frac{\pi}{2} \left( \frac{2^{\frac{7}{10}} 2^{\bar{\pi}^7}}{\Gamma\left(\frac{3}{10}\right)} + \frac{2^{\frac{13}{10}} 2^{\bar{\pi}^{13}}}{\Gamma\left(\frac{13}{10}\right)} - \frac{2^{\frac{7}{10}} 2^{\bar{\pi}^7}}{\Gamma\left(\frac{13}{10}\right)} \right) \csc\left(\frac{7\pi}{10}\right) + O\left(\bar{t}^{\frac{27}{10}}\right). \quad (E.8) \]
Also,
\[ e^\bar{t} = 1 + \bar{t}^{\frac{1}{2}} + O\left(\bar{t}^3\right), \]
therefore
\[ e^\bar{t} \bar{t}^{\frac{7}{10}} = \bar{t}^{\frac{7}{10}} + \frac{1}{2} \bar{t}^{\frac{17}{10}} + O\left(\bar{t}^{\frac{27}{10}}\right). \quad (E.9) \]
Thus,
\[ e^\bar{t} \bar{t}^{\frac{7}{10}} \left( I_{\frac{-3}{10}}(\bar{t}) + I_{\frac{7}{10}}(\bar{t}) \right) = \left( \bar{t}^{\frac{7}{10}} + \bar{t}^{\frac{17}{10}} + \frac{1}{2} \bar{t}^{\frac{27}{10}} \right) \left\{ \frac{2^{\frac{3}{10}} 2^{\bar{\pi}^3}}{\Gamma\left(\frac{7}{10}\right)} + \frac{2^{\frac{17}{10}} 2^{\bar{\pi}^7}}{\Gamma\left(\frac{17}{10}\right)} + \frac{2^{\frac{3}{10}} 2^{\bar{\pi}^3}}{\Gamma\left(\frac{17}{10}\right)} \right\} \]
\[ + O\left(\bar{t}^{\frac{27}{10}}\right), \]
\[ = \frac{2^{\frac{3}{10}} 2^{\bar{\pi}^3}}{\Gamma\left(\frac{7}{10}\right)} + \frac{2^{\frac{17}{10}} 2^{\bar{\pi}^7}}{\Gamma\left(\frac{17}{10}\right)} + \frac{2^{\frac{3}{10}} 2^{\bar{\pi}^3}}{\Gamma\left(\frac{17}{10}\right)} + O\left(\bar{t}^{\frac{12}{10}}\right), \]
\[ = \frac{2^{\frac{3}{10}} 2^{\bar{\pi}^3}}{\Gamma\left(\frac{7}{10}\right)} + \frac{\frac{19}{2} 2^{\frac{3}{10}} 2^{\bar{\pi}^3}}{\Gamma\left(\frac{7}{10}\right)} + O\left(\bar{t}^{\frac{12}{10}}\right), \quad (E.10) \]
and

\[ e^{\tilde{t} \tilde{m}} \left( K_{\frac{\pi}{m}}(\tilde{t}) - K_{\frac{\pi}{m}}(\tilde{t}) \right) = \left( e^{\text{i} \frac{\pi}{m}} + e^{\text{i} \frac{\pi}{m}} + 2 e^{\text{i} \frac{\pi}{m}} \right) \left\{ \frac{\pi}{2} \left( \frac{2^{9/10} \tilde{t}^{\pi}}{\Gamma \left( \frac{7}{10} \right)} + \frac{2^{17/10} \tilde{t}^{\pi}}{\Gamma \left( \frac{13}{10} \right)} - \frac{2^{3/10} \tilde{t}^{\pi}}{\Gamma \left( \frac{13}{10} \right)} \right) \csc \left( \frac{3\pi}{10} \right) \\
- \frac{\pi}{2} \left( \frac{2^{9/10} \tilde{t}^{\pi}}{\Gamma \left( \frac{7}{10} \right)} + \frac{2^{17/10} \tilde{t}^{\pi}}{\Gamma \left( \frac{13}{10} \right)} - \frac{2^{3/10} \tilde{t}^{\pi}}{\Gamma \left( \frac{13}{10} \right)} \right) \csc \left( \frac{7\pi}{10} \right) \right\} + \mathcal{O} \left( \tilde{t}^{\frac{12}{5}} \right), \]

\[ = \frac{\pi}{2} \csc \left( \frac{3\pi}{10} \right) \left( e^{\text{i} \frac{\pi}{m}} + e^{\text{i} \frac{\pi}{m}} + 2 e^{\text{i} \frac{\pi}{m}} \right) \left\{ \frac{2\tilde{t}^{\pi}}{\Gamma \left( \frac{3}{10} \right)} + \frac{2^{9/10} \tilde{t}^{\pi}}{\Gamma \left( \frac{7}{10} \right)} - \frac{2^{3/10} \tilde{t}^{\pi}}{\Gamma \left( \frac{13}{10} \right)} + \frac{2^{3/10} \tilde{t}^{\pi}}{\Gamma \left( \frac{13}{10} \right)} \right\} + \mathcal{O} \left( \tilde{t}^{\frac{12}{5}} \right), \]

\[ = \frac{\pi}{2} \csc \left( \frac{3\pi}{10} \right) \left\{ \frac{2\tilde{t}^{\pi}}{\Gamma \left( \frac{3}{10} \right)} + \left( \frac{10}{3} \tilde{t}^{\pi} + 2 \tilde{t}^{\pi} \right) \tilde{t} + \left( \frac{10}{3} \tilde{t}^{\pi} + 2 \tilde{t}^{\pi} \right) \tilde{t}^{2} \right\} + \mathcal{O} \left( \tilde{t}^{\frac{12}{5}} \right), \]

\[ = - \frac{\pi}{2} \frac{\csc \left( \frac{3\pi}{10} \right)}{\Gamma \left( \frac{3}{10} \right)} \left\{ 1 + \frac{8}{3} \tilde{t} + 3 \tilde{t}^{2} \right\} + \mathcal{O} \left( \tilde{t}^{\frac{12}{5}} \right), \]

\[ = - \frac{\pi}{2} \frac{\tilde{t}^{\pi}}{\Gamma \left( \frac{3}{10} \right)} \left\{ 1 + \frac{12}{7} \tilde{t} + \mathcal{O} \left( \tilde{t}^{\frac{12}{5}} \right) \right\}. \]
Substituting Equations (E.10) and (E.11) into Equation (E.2) gives

\[
x(\hat{t}) = \hat{A}\left(\frac{2\hat{\pi}\hat{t}^2}{\Gamma\left(\frac{7}{10}\right)} + \frac{12\hat{\pi}^3\hat{t}^2}{\Gamma\left(\frac{7}{10}\right)}\right) \\
- \hat{B}\frac{\hat{\pi}2\hat{\pi}\text{cosec}\left(\frac{3\pi}{10}\right)}{\Gamma\left(\frac{3}{10}\right)} \left\{1 + \frac{8}{3}\hat{t} + 3\hat{t}^2\right\} \\
+ \hat{B}\frac{\hat{\pi}\text{cosec}\left(\frac{3\pi}{10}\right)}{\Gamma\left(\frac{7}{10}\right)} \left\{1 + \frac{12}{7}\hat{t}\right\} + \mathcal{O}\left(\hat{t}^{12}\right), \\
= \frac{2\hat{\pi}\hat{t}^2}{\Gamma\left(\frac{7}{10}\right)} \left(\frac{\pi}{2}\text{cosec}\left(\frac{3\pi}{10}\right) \hat{B} + \hat{A}\right) \left\{1 + \frac{12}{7}\hat{t}\right\} \\
- \hat{B}\frac{\hat{\pi}2\hat{\pi}\text{cosec}\left(\frac{3\pi}{10}\right)}{\Gamma\left(\frac{3}{10}\right)} \left\{1 + \frac{8}{3}\hat{t} + 3\hat{t}^2\right\},
\]

(E.12)

which on re-labelling the constants becomes

\[
x(\hat{t}) = \hat{A}\hat{t}^2 \left\{1 + \frac{12}{7}\hat{t}\right\} + \hat{B} \left\{1 + \frac{8}{3}\hat{t} + 3\hat{t}^2\right\}.
\]

(E.13)

Which is the same as Equation (6.69). Therefore it has been shown that for the second order ODE given by Equation (6.30) the second order approximation obtained using Frobenius’ technique is identical to the second order approximation of the analytical solution.
The analytic solution to Equation (6.26) is given by Equation (6.27). Using the definition of the hypergeometric function as given in Equation (6.29) it can be seen that a second order approximation for a hypergeometric function is given by

\[
pF_q(a, b, z) = \sum_{n=0}^{\infty} \frac{z^n}{n!} \frac{\prod_{i=1}^{p} \Gamma(a_i+n)}{\prod_{j=1}^{q} \Gamma(b_j+n)},
\]

(E.14)

Therefore an approximation to Equation (6.27) is given by

\[
N_{G_1}(t) = C_1(e^{-2t})^{1-\frac{i}{2}} \left\{ 1 - \frac{2^\frac{i}{4}}{(2 - 2^\frac{i}{4})^4 - 2} (e^{-2t}) - \frac{(2 - 2^\frac{i}{4})^3 2^\frac{i}{4}}{(2 - 2^\frac{i}{4})^4 - 2} \left\{ (4 - 2^\frac{i}{4})^4 - 2 \right\} (e^{-2t})^2 \right\}
+ C_2(e^{-2t})^{1+\frac{i}{2}} \left\{ 1 + \frac{2^\frac{i}{4}}{(2 + 2^\frac{i}{4})^4 - 2} (e^{-2t}) + \frac{(2 + 2^\frac{i}{4})^3 2^\frac{i}{4}}{(2 + 2^\frac{i}{4})^4 - 2} \left\{ (4 + 2^\frac{i}{4})^4 - 2 \right\} (e^{-2t})^2 \right\}
+ C_3(e^{-2t})^{1-\frac{i}{2}} \left\{ 1 + \frac{2^\frac{i}{4} i}{(2 - 2^\frac{i}{4} i)^4 - 2} (e^{-2t}) + \frac{(2 - 2^\frac{i}{4} i)^3 2^\frac{i}{4} i}{(2 - 2^\frac{i}{4} i)^4 - 2} \left\{ (4 - 2^\frac{i}{4} i)^4 - 2 \right\} (e^{-2t})^2 \right\}
+ C_4(e^{-2t})^{1+\frac{i}{2}} \left\{ 1 - \frac{2^\frac{i}{4} i}{(2 + 2^\frac{i}{4} i)^4 - 2} (e^{-2t}) - \frac{(2 + 2^\frac{i}{4} i)^3 2^\frac{i}{4} i}{(2 + 2^\frac{i}{4} i)^4 - 2} \left\{ (4 + 2^\frac{i}{4} i)^4 - 2 \right\} (e^{-2t})^2 \right\}
+ \mathcal{O}(e^{-6t}).
\]

(E.15)

Since the parameter \( \lambda \), has a value of two, the solution given by Equations (6.94 and 6.95), may be written as

\[
N_{G_1}(\tilde{t}) = P(\tilde{t}) + \mathcal{O}(\tilde{t}^2),
\]

(E.16)
where

$$P(t) = \sum_{i=1}^{4} a_i \tilde{r}_i \left\{ 1 + \frac{(2r_i - 1)^3}{(2r_i + 1)^4 - 2} \tilde{t} + \frac{(2r_i + 1)^3(2r_i - 1)^3}{((2r_i + 3)^4 - 2)((2r_i + 1)^4 - 2)} \tilde{t}^2 \right\}, \quad (E.17)$$

where the $i$ correspond to the four different values of $r$. Upon making the substitution given in Equation (6.74) to repose Equations (E.16 and E.17) in terms of $t$ it can be seen that Equations (E.16 and E.17) are identical to Equation (E.15). Thus, the approximation obtained using Frobenius’ technique is identical to the first order approximation of the analytical solution.
Appendix F

Case I for a Cytotoxic Drug-Cell Cycle Model When Phase Rate Constants Are Not Equal

The equations governing a population of cells with the effects of drug interaction are given by Equations (6.1), the corresponding equation for drug concentration is given by Equation (6.2). As detailed in Section 6.1 these equations may be simplified by making certain assumptions about the model.

In this appendix these assumptions are re-stated, with the assumption $k_1(D(t)) = k_2(D(t)) = k_3(D(t)) = k_4(D(t)) = k$ being removed. However, it is assumed that the phase rates are constant and not dependent on the drug concentration, $D(t)$. Case I for a cytotoxic drug interaction is then analysed for this more general case.

By assuming the drug is delivered in a single, bolus dose $d_m(t)$ may be incorporated into the initial conditions. The rate of clearance may be assumed to be directly proportional to the amount of drug in the system. Furthermore, the drug can be assumed to be taken up equally by all cells, regardless of phase. The rate the drug is used up is clearly proportional to the total cell population density. Also, if the concentration of the drug in the system is not so high that the cells are being saturated with the amount of drug they may take up then the rate the drug is used is first order with respect to the drug concentration thus $g(N_{G_1}(t), N_{S}(t), N_{G_2}(t), N_M(t), D(t)) = \delta N_T(t)D(t)$ where $N_T(t)$ is
the total cell population density at time $t$. Equation (6.2) now becomes

$$\frac{dD(t)}{dt} = -d_{\text{out}}D(t) - \delta N_T(t)D(t). \quad (F.1)$$

For a cytotoxic drug the same assumptions as in Section 6.2 are made. That is, the drug only effects cells in the $S$ phase, thus $f_1(D(t)) = f_3(D(t)) = f_4(D(t)) = 0$. Furthermore, let the drug effect cells in the $S$ phase in a way which is directly proportional to the drug concentration in the inter cellular media, hence $f_2(D(t)) = eD(t)$ where $e$ can be thought of as the efficacy of the drug. Since the drug has a cytotoxic effect only the average number of cells produced at each division is not dependent on the drug concentration and is therefore a constant, thus $P(D(t)) = 2$. Equations (6.1) now simplify to

$$\frac{dN_{G_1}(t)}{dt} = 2k_1N_M(t) - k_2N_{G_1}(t),$$

$$\frac{dN_S(t)}{dt} = k_2N_{G_1}(t) - k_3N_S(t) - eD(t)N_S(t),$$

$$\frac{dN_{G_2}(t)}{dt} = k_3N_S(t) - k_4N_{G_2}(t),$$

$$\frac{dN_M(t)}{dt} = k_4N_{G_2}(t) - k_1N_M(t). \quad (F.2)$$

Equations (F.1) and (F.2) together with suitable initial conditions now represent a simplified model with a cytotoxic drug interaction where the phase constants, $k_i, i \in \{G_1, S, G_2, M\}$, are not equal. It should be noted by setting $k_1 = k_2 = k_3 = k_4 = k$ Equation (F.2) simply becomes (6.6). The initial conditions are chosen to be the same as that for the case considered in Section 6.2 where the phase rates are all equal and are given by Equations (6.7) and (6.8).

**F.1 Non-dimensionalisation of the Equations**

As discussed in Section 6.2.1 it is helpful to non-dimensionalise the system of equations given by Equations F.2 and (F.1). The first step in the non-dimensionalisation process is to introduce new, dimensionless variables $\tilde{t}$, $\tilde{D}(\tilde{t})$, $\tilde{N}_{G_1}(\tilde{t})$, $\tilde{N}_S(\tilde{t})$, $\tilde{N}_{G_2}(\tilde{t})$, $\tilde{N}_M(\tilde{t})$ and
\( \tilde{N}_T(\tilde{t}) \) defined as

\[
\tilde{t} = \frac{t}{b},
\]

\[
\tilde{N}_{G1}(\tilde{t}) = \frac{N_{G1}(t)}{a},
\]

\[
\tilde{N}_S(\tilde{t}) = \frac{N_S(t)}{a},
\]

\[
\tilde{N}_{G2}(\tilde{t}) = \frac{N_{G2}(t)}{a},
\]

\[
\tilde{N}_M(\tilde{t}) = \frac{N_M(t)}{a},
\]

\[
\tilde{N}_T(\tilde{t}) = \sum_i \tilde{N}_i(\tilde{t}),
\]

\[
\tilde{D}(\tilde{t}) = \frac{D(t)}{c}.
\]

Where \( a, b \) and \( c \) are dimensional constants which may be arbitrarily chosen as to simplify the system. Note, the cell densities in the different compartments are all scaled equally to avoid potentially misleading distributions. Substituting the non-dimensional variables into Equations (F.1) and (F.2) gives

\[
\frac{a}{b} \frac{d\tilde{N}_{G1}(\tilde{t})}{dt} = 2ak_1\tilde{N}_M(\tilde{t}) - ak_2\tilde{N}_{G1}(\tilde{t}),
\]

\[
\frac{a}{b} \frac{d\tilde{N}_S(\tilde{t})}{dt} = ak_2\tilde{N}_{G1}(\tilde{t}) - ak_3\tilde{N}_S(\tilde{t}) - ace\tilde{D}(\tilde{t})\tilde{N}_S(\tilde{t}),
\]

\[
\frac{a}{b} \frac{d\tilde{N}_{G2}(\tilde{t})}{dt} = ak_3\tilde{N}_S(\tilde{t}) - ak_4\tilde{N}_{G2}(\tilde{t}),
\]

\[
\frac{a}{b} \frac{d\tilde{N}_M(\tilde{t})}{dt} = ak_4\tilde{N}_{G2}(\tilde{t}) - ak_1\tilde{N}_M(\tilde{t}),
\]

and

\[
\frac{c}{b} \frac{d\tilde{D}(\tilde{t})}{dt} = -cd_{out}\tilde{D}(\tilde{t}) - \delta ac\tilde{N}_T(\tilde{t})\tilde{D}(\tilde{t}).
\]
The corresponding initial conditions are now given by

\[ \tilde{N}_i(0) = \frac{\tilde{N}_T(0)}{4} \text{ where } i \in \{G_1, S, G_2, M\}, \]  

(F.6)

and

\[ \tilde{D}(0) = \tilde{D}_I \text{ where } \tilde{D}_I = \frac{D_I}{c}. \]  

(F.7)

Equations (F.4) and (F.5) may be re-arranged to give, upon dropping the tilde notation,

\[ \frac{dN_{G_1}(t)}{dt} = 2bk_1N_M(t) - bk_2N_{G_1}(t), \]  

\[ \frac{dN_S(t)}{dt} = bk_2N_{G_1}(t) - bk_3N_S(t) - bceD(t)N_S(t), \]  

(F.8)

\[ \frac{dN_{G_2}(t)}{dt} = bk_3N_S(t) - bk_4N_{G_2}(t), \]

\[ \frac{dN_M(t)}{dt} = bk_4N_{G_2}(t) - bk_1N_M(t), \]

and

\[ \frac{dD(t)}{dt} = -bd_{out}D((t) - \delta abN_T(t)D(t). \]  

(F.9)

The constants \( b \) and \( c \) are now chosen as \( b = \frac{1}{k_1} \) and \( c = \frac{k_1}{e} \) thus simplifying Equations (F.8) and (F.9) to

\[ \frac{dN_{G_1}(t)}{dt} = 2N_M(t) - \frac{k_2}{k_1}N_{G_1}(t), \]

\[ \frac{dN_S(t)}{dt} = \frac{k_2}{k_1}N_{G_1}(t) - \frac{k_3}{k_1}N_S(t) - D(t)N_S(t), \]  

(F.10)

\[ \frac{dN_{G_2}(t)}{dt} = \frac{k_3}{k_1}N_S(t) - \frac{k_4}{k_1}N_{G_2}(t), \]

\[ \frac{dN_M(t)}{dt} = \frac{k_4}{k_1}N_{G_2}(t) - N_M(t), \]

and

\[ \frac{dD(t)}{dt} = -\frac{d_{out}}{k_1}D((t) - \frac{\delta a}{k_1}N_T(t)D(t). \]  

(F.11)

The parameter \( a \) may be chosen arbitrarily, but a sensible choice is \( a = \frac{N_T(0)}{4} \). This value is chosen such that the normalised initial conditions for the cell population densities in
each of the four compartments are all one. It should be noted that \( a \gg 1 \). The parameters \( d_{\text{out}} \) and \( k \) are both \( \mathcal{O}(1) \) as one represents the clearance of the drug per unit time and the other the rate at which cells progress between the compartments. The parameter \( \delta \) represents the amount of drug absorbed per cell per unit time and as such may be assumed to be very small, \( \delta \ll 1 \). Five new parameters are now introduced; \( A = \frac{k_2}{k_1} \), \( B = \frac{k_3}{k_1} \), \( C = \frac{k_4}{k_1} \), \( \lambda = \frac{d_{\text{out}}}{k_1} \) and \( \epsilon = \frac{a}{k_1} \). The order of \( A \), \( B \), \( C \) and \( \lambda \) are all \( \mathcal{O}(1) \) and the order of \( \epsilon \) is unknown. Equations (F.10) and (F.11) may now be rewritten as

\[
\frac{dN_{G_1}(t)}{dt} = 2N_M(t) - AN_{G_1}(t),
\]

\[
\frac{dN_S(t)}{dt} = AN_{G_1}(t) - BN_S(t) - D(t)N_S(t),
\]

\[
\frac{dN_{G_2}(t)}{dt} = BN_S(t) - CN_{G_2}(t),
\]

\[
\frac{dN_M(t)}{dt} = CN_{G_2}(t) - N_M(t),
\]

and

\[
\frac{dD(t)}{dt} = -\lambda D(t) - \epsilon N_T(t)D(t),
\]

respectively.

### F.2 Obtaining an Approximate Solution

For the case \( \epsilon \ll 1 \) the process for obtaining a solution to the system of equations given by Equations (F.12) and (F.13) is the same as that described in Section 6.2.2. As such the description of the process here is brief, for a more detailed discussion see Section 6.2.2.

Equation (F.13) decouples from the system and may readily be solved. This solution may then be put back into the remaining cell population equations, given by Equations (F.12), resulting in a system of four first order ODEs, which may then be converted into a single
fourth order ODE, given by
\[
\frac{d^4 N_{G_1}(t)}{dt^4} + \left( A + B + C + 1 + D_1 e^{-\Delta t} \right) \frac{d^3 N_{G_1}(t)}{dt^3} \\
+ \left( A + AB + AC + B + BC + C + (A + C + 1)D_1 e^{-\Delta t} \right) \frac{d^2 N_{G_1}(t)}{dt^2} \\
+ \left( AB + ABC + AC + BC + (A + AC + C)D_1 e^{-\Delta t} \right) \frac{dN_{G_1}(t)}{dt} \\
- \left( ABC - ACD_1 e^{-\Delta t} \right) N_{G_1}(t) = 0.
\] (F.14)

It can readily be seen that if \( A = B = C = 1 \) then Equation (F.14) simplifies to Equation (6.26). For ease of notation the new parameters \( \Gamma = A + B + C + 1, \Delta = A + AB + AC + B + BC + C, E = A + C + 1, Z = AB + ABC + AC + BC \) and \( H = A + AC + C \) are introduced. Applying the change of variables given in Equation (6.74) to Equation (F.14) yields
\[
\lambda^4 \frac{d^4 N_{G_1}(\hat{t})}{d\hat{t}^4} + \left\{ 6\lambda^4 \hat{t}^3 - \Gamma \lambda^3 \hat{t}^3 - \lambda^3 \hat{t}^4 \right\} \frac{d^3 N_{G_1}(\hat{t})}{d\hat{t}^3} \\
+ \left\{ 7\lambda^4 \hat{t}^2 - 3\Gamma \lambda^3 \hat{t}^2 - 3\lambda^3 \hat{t}^3 + \Delta \lambda^2 \hat{t}^2 + E \lambda^2 \hat{t}^3 \right\} \frac{d^2 N_{G_1}(\hat{t})}{d\hat{t}^2} \\
+ \left\{ \lambda^4 \hat{t} - \Gamma \lambda^3 \hat{t} - \lambda^3 \hat{t}^2 + \Delta \lambda^2 \hat{t} + E \lambda^2 \hat{t}^2 - Z \lambda \hat{t} - H \lambda \hat{t}^2 \right\} \frac{dN_{G_1}(\hat{t})}{d\hat{t}} \\
+ \left( AC \hat{t} - ABC \right) N_{G_1}(\hat{t}) = 0.
\] (F.15)

Equation (F.15) is singular at \( \hat{t} = 0 \) and is of the form given in Equation (6.76) where
\[
P(\hat{t}) = \lambda^4 \hat{t}^4, \quad \text{(F.16a)}
\]
\[
Q(\hat{t}) = 6\lambda^4 \hat{t}^3 - \Gamma \lambda^3 \hat{t}^3 - \lambda^3 \hat{t}^4, \quad \text{(F.16b)}
\]
\[
R(\hat{t}) = 7\lambda^4 \hat{t}^2 - 3\Gamma \lambda^3 \hat{t}^2 - 3\lambda^3 \hat{t}^3 + \Delta \lambda^2 \hat{t}^2 + E \lambda^2 \hat{t}^3, \quad \text{(F.16c)}
\]
\[
S(\hat{t}) = \lambda^4 \hat{t} - \Gamma \lambda^3 \hat{t} - \lambda^3 \hat{t}^2 + \Delta \lambda^2 \hat{t} + E \lambda^2 \hat{t}^2 - Z \lambda \hat{t} - H \lambda \hat{t}^2, \quad \text{(F.16d)}
\]
\[
T(\hat{t}) = (AC \hat{t} - ABC). \quad \text{(F.16e)}
\]
For $\tilde{t} = 0$ to be a regular singularity $\lim_{\tilde{t} \to 0} \frac{i Q(\tilde{t})}{P(\tilde{t})}$, $\lim_{\tilde{t} \to 0} \frac{i^2 R(\tilde{t})}{P(\tilde{t})}$, $\lim_{\tilde{t} \to 0} \frac{i^3 S(\tilde{t})}{P(\tilde{t})}$ and $\lim_{\tilde{t} \to 0} \frac{i^4 T(\tilde{t})}{P(\tilde{t})}$ must all remain finite. Using the expressions from Equations (F.16) yields

$$\lim_{\tilde{t} \to 0} \frac{i Q(\tilde{t})}{P(\tilde{t})} = \lim_{\tilde{t} \to 0} \frac{i \{6\lambda^4 \tilde{t}^3 - \Gamma \lambda^3 \tilde{t}^2 - \lambda^3 \tilde{t}^4 \}}{\lambda^4 \tilde{t}^4},$$

$$= 6 - \lim_{\tilde{t} \to 0} \left( \frac{\Gamma + \tilde{t}}{\lambda} \right),$$

$$= \frac{1}{\lambda} (6\lambda - \Gamma), \quad (F.17)$$

$$\lim_{\tilde{t} \to 0} \frac{i^2 R(\tilde{t})}{P(\tilde{t})} = \lim_{\tilde{t} \to 0} \frac{i^2 \{7\lambda^4 \tilde{t}^2 - 3\Gamma \lambda^3 \tilde{t}^2 - 3\lambda^3 \tilde{t}^3 + \Delta \lambda^2 \tilde{t}^2 + E \lambda^2 \tilde{t}^3 \}}{\lambda^4 \tilde{t}^4},$$

$$= 7 + \frac{\Delta - 3\lambda \Gamma}{\lambda^2} + \lim_{\tilde{t} \to 0} \frac{i(E - 3\lambda)}{\lambda^2},$$

$$= 7 + \frac{\Delta - 3\lambda \Gamma}{\lambda^2}, \quad (F.18)$$

$$\lim_{\tilde{t} \to 0} \frac{i^3 S(\tilde{t})}{P(\tilde{t})} = \lim_{\tilde{t} \to 0} \frac{i^3 \{\lambda^4 \tilde{t} - \Gamma \lambda^3 \tilde{t} - \lambda^3 \tilde{t}^2 + \Delta \lambda^2 \tilde{t} + E \lambda^2 \tilde{t}^2 - Z \lambda \tilde{t} - H \lambda^2 \}}{\lambda^4 \tilde{t}^4},$$

$$= 1 - \lim_{\tilde{t} \to 0} \left( \frac{\Gamma + \tilde{t}}{\lambda} + \frac{\Delta + E \tilde{t}}{\lambda^2} - \frac{Z + H \tilde{t}}{\lambda^3} \right),$$

$$= 1 - \frac{\Gamma}{\lambda} + \frac{\Delta}{\lambda^2} - \frac{Z}{\lambda^3}, \quad (F.19)$$

$$\lim_{\tilde{t} \to 0} \frac{i^4 T(\tilde{t})}{P(\tilde{t})} = \lim_{\tilde{t} \to 0} \frac{i^4 (AC \tilde{t} - ABC)}{\lambda^4 \tilde{t}^4},$$

$$= \lim_{\tilde{t} \to 0} \frac{AC \tilde{t} - ABC}{\lambda^4},$$

$$= -\frac{ABC}{\lambda^4}. \quad (F.20)$$
Hence the singularity at \( \tilde{t} = 0 \) is regular. Because the singularity is regular, Equation (F.15) may be solved using the same technique detailed in Section 6.2.2.

Substituting these expressions for \( N_{G1}(\tilde{t}) \), and its derivatives given by Equations (6.82) and (6.83) into Equation (F.15) and proceeding as in Section 6.2.2 gives

\[
\lambda^4 \sum_{n=0}^{\infty} a_n \Phi^n \Phi_{-1} \Phi_{-2} \tilde{\Phi}_n^3 + \{6\lambda^4 - \Gamma \lambda^3 \} \sum_{n=0}^{\infty} a_n \Phi^n \Phi_{-1} \Phi_{-2} \tilde{\Phi}_n^2 \\
- \lambda^3 \sum_{n=0}^{\infty} a_n \Phi^n \Phi_{-1} \Phi_{-2} \tilde{\Phi}_n^2 + \{7\lambda^4 - 3\Gamma \lambda^3 + \Delta \lambda^2 \} \sum_{n=0}^{\infty} a_n \Phi^n \Phi_{-1} \tilde{\Phi}_n + Z \sum_{n=0}^{\infty} a_n \Phi^n \tilde{\Phi}_n \\
+ \{E \lambda^2 - 3\lambda^3 \} \sum_{n=0}^{\infty} a_n \Phi^n \Phi_{-1} \tilde{\Phi}_n + \{\lambda^4 - \Gamma \lambda^3 + \Delta \lambda^2 \} \sum_{n=0}^{\infty} a_n \Phi^n \tilde{\Phi}_n - Z \lambda \sum_{n=0}^{\infty} a_n \Phi^n \tilde{\Phi}_n
\]

where \( \Phi \) is defined in Equation (6.40). Equation (F.21) may be rewritten as

\[
[\lambda^4 r^4 - \Gamma \lambda^3 r^3 + \Delta \lambda^2 r^2 - Z \lambda r - ABC] a_0 \tilde{r} \\
+ \sum_{n=1}^{\infty} \{[\lambda^4 (\Phi_0)^4 - \Gamma \lambda^3 (\Phi_0)^3 + \Delta \lambda^2 (\Phi_0)^2 - Z \lambda \Phi_0 - ABC] a_n \\
+ \{\lambda^3 (\Phi_0)^3 + \{E \lambda^2 + 3\lambda^3 \} (\Phi_0)^2 - \{2E \lambda^2 + 3\lambda^3 + H \lambda \} \Phi_0^n \}
+ \lambda^3 + E \lambda^2 + H \lambda + AC \} a_{n-1} \} \tilde{\Phi}_n = 0.
\]

(F.21)

If Equation (F.22) is to satisfied then the coefficient for each power of \( \tilde{t} \) must equal zero. Considering the coefficient of the \( \tilde{r} \) term gives

\[
[\lambda^4 r^4 - \Gamma \lambda^3 r^3 + \Delta \lambda^2 r^2 - Z \lambda r - ABC] a_0 = 0.
\]

(F.23)

Since \( a_0 \neq 0 \) it is clear \( r \) satisfies the quartic equation

\[
\lambda^4 r^4 - \Gamma \lambda^3 r^3 + \Delta \lambda^2 r^2 - Z \lambda r - ABC = 0.
\]

(F.24)
For general \( A, B \) and \( C \) this quartic does not have a simple solution, however an analytic solution may be obtained using standard techniques for quartic polynomial. Let the roots of this equation be given by \( R_i \), \( i \in \{1, 2, 3, 4\} \). In only a few specific cases will there be multiple roots or roots differing by an integer. It is therefore assumed this is not the case and the roots are all distinct and do not differ by an integer, thus ensuring all four solutions are linearly independent. Since Equation (F.15) is linear in \( N_{G1}(\hat{t}) \) the general solution will be given by

\[
N_{G1}(\hat{t}) = c_1x_1(\hat{t}) + c_2x_2(\hat{t}) + c_3x_3(\hat{t}) + c_4x_4(\hat{t}) = 0, \tag{F.25}
\]

where \( x_i(\hat{t}) \) is the solution corresponding to \( R_i \).

For the coefficients of \( \hat{t}^n \) for \( n \geq 1 \) to equal zero the recurrence relation

\[
a_n = \left( \frac{\mathcal{R}}{\mathcal{D}} \right) a_{n-1}, \tag{F.26}
\]

where

\[
\mathcal{R} = \lambda^3 (\Phi_0^n)^3 - \{E\lambda^2 + 3\lambda^3\} (\Phi_0^n)^2 + \{2E\lambda^2 + 3\lambda^3 + H\lambda\} \Phi_0^n
\]

\[
- \lambda^3 - E\lambda^2 - H\lambda - AC,
\]

and

\[
\mathcal{D} = \lambda^4 (\Phi_0^n)^4 - \Gamma\lambda^3 (\Phi_0^n)^3 + \Delta\lambda^2 (\Phi_0^n)^2 - Z\lambda\Phi_0^n - ABC,
\]

must be satisfied. As before, to check the resulting series converge the ratio test may be implemented. It can be seen that the ratio of two successive terms is given by

\[
L = \lim_{n \to \infty} \left| \frac{a_n \hat{t}^n \Phi_0^n}{a_{n-1} \hat{t}^{n+r-1}} \right|,
\]

\[
= \lim_{n \to \infty} \left| \frac{\left( \frac{\mathcal{R}}{\mathcal{D}} \right) a_{n-1} \hat{t}^{n-1}}{a_{n-1}} \right|,
\]

\[
= \lim_{n \to \infty} \left| \left( \frac{\mathcal{R}}{\mathcal{D}} \right) \hat{t} \right|,
\]

\[
= 0.
\]

Since \( L < 1 \) the series converges absolutely for all \( \hat{t} \). In order to check the validity of this approximation on the solution it is possible to compare this result with a standard
numerical discretisation approach. To do this it is necessary to choose suitable parameters and initial conditions. The initial conditions are chosen to be those given by Equations (6.23), (6.24) and (6.25) and the values of the parameters, $k_i$, for $i \in \{1, 2, 3, 4\}$, $D_I$ and $\lambda$ are chosen as

\begin{align*}
k_1 &= 2, \quad \text{(F.30a)} \\
k_2 &= \frac{1}{10}, \quad \text{(F.30b)} \\
k_3 &= \frac{1}{5}, \quad \text{(F.30c)} \\
k_4 &= \frac{1}{3}, \quad \text{(F.30d)} \\
D_I &= 1, \quad \text{(F.30e)} \\
\lambda &= 2. \quad \text{(F.30f)}
\end{align*}

From Equations (F.30) the values

\begin{align*}
A &= \frac{1}{20}, \quad \text{(F.31a)} \\
B &= \frac{1}{10}, \quad \text{(F.31b)} \\
C &= \frac{1}{6}. \quad \text{(F.31c)}
\end{align*}

are readily obtained. Using these values Equation (F.24) becomes

\begin{equation}
16r^4 - \frac{158}{15}r^3 + \frac{104}{75}r^2 - \frac{37}{600}r - \frac{1}{1200} = 0, \quad \text{(F.32)}
\end{equation}

which has the solutions

\begin{align*}
R_1 &= 0.5012, \quad \text{(F.33a)} \\
R_2 &= -0.0107, \quad \text{(F.33b)} \\
R_3 &= 0.0839 + 0.0515i, \quad \text{(F.33c)} \\
R_4 &= 0.0839 - 0.0515i. \quad \text{(F.33d)}
\end{align*}
Since these four values are distinct and do not differ by an integer the four corresponding solutions are linearly independent, meaning the full solution to Equation (F.14) is now given by Equation (F.25). For a first approximation to Equation (F.25) let

\[ x_\ell (\hat{\ell}) = P_\ell (\hat{\ell}) + \mathcal{O}(\ell^2), \]

(F.34)

where

\[ P_\ell (\hat{\ell}) = C_\ell \left\{ a_{i_0} + a_{i_1} \hat{\ell} \right\}, \]

\[ = C_\ell a_{i_0} \left\{ 1 + \frac{8 \hat{R}_i^3 - \frac{433}{15} \hat{R}_i^2 + \frac{2051}{60} \hat{R}_i - \frac{533}{40}}{16 \hat{R}_i^4 - \frac{158}{15} \hat{R}_i^3 + \frac{104}{75} \hat{R}_i^2 - \frac{37}{600} \hat{R}_i - \frac{1}{1200} \hat{\ell}} \right\}, \]

(F.35)

\[ = \hat{C}_\ell \left\{ 1 + \frac{8 \hat{R}_i^3 - \frac{433}{15} \hat{R}_i^2 + \frac{2051}{60} \hat{R}_i - \frac{533}{40}}{16 \hat{R}_i^4 - \frac{158}{15} \hat{R}_i^3 + \frac{104}{75} \hat{R}_i^2 - \frac{37}{600} \hat{R}_i - \frac{1}{1200} \hat{\ell}} \right\}, \]

for \( \ell = e \{ 1, 3, 4 \}, \) where \( \hat{R}_i = 1 + R_i \) and

\[ P_2 (\hat{\ell}) = C_2 \left\{ a_{i_0} + a_{i_1} \hat{\ell} + a_{i_2} \hat{\ell}^2 \right\}, \]

\[ = \hat{C}_2 \left\{ a_{i_0} + a_{i_1} \hat{\ell} + a_{i_2} \hat{\ell}^2 \right\}, \]

(F.36)

where

\[ a_{i_1} = \frac{8 \hat{R}_i^3 - \frac{433}{15} \hat{R}_i^2 + \frac{2051}{60} \hat{R}_i - \frac{533}{40}}{16 \hat{R}_i^4 - \frac{158}{15} \hat{R}_i^3 + \frac{104}{75} \hat{R}_i^2 - \frac{37}{600} \hat{R}_i - \frac{1}{1200}}, \]

(F.37)

and

\[ a_{i_2} = \hat{V} \hat{V}, \]

(F.38)

with

\[ \hat{V} = \frac{8 \hat{R}_i^3 - \frac{433}{15} \hat{R}_i^2 + \frac{2051}{60} \hat{R}_i - \frac{533}{40}}{16 \hat{R}_i^4 - \frac{158}{15} \hat{R}_i^3 + \frac{104}{75} \hat{R}_i^2 - \frac{37}{600} \hat{R}_i - \frac{1}{1200}}, \]

(F.39)

and

\[ \hat{V} = \frac{8 \left( 1 + \hat{R}_2 \right)^3 - \frac{433}{15} \left( 1 + \hat{R}_2 \right)^2 + \frac{2051}{60} \left( 1 + \hat{R}_2 \right) - \frac{533}{40}}{16 \left( 1 + \hat{R}_2 \right)^4 - \frac{158}{15} \left( 1 + \hat{R}_2 \right)^3 + \frac{104}{75} \left( 1 + \hat{R}_2 \right)^2 - \frac{37}{600} \hat{R}_2 - \frac{1}{1200}}. \]

(F.40)
It should be noted it is necessary to approximate $P_2(\tilde{t})$ with three terms since $\Re(\epsilon)(t_2) < 0$. For $P_i(\tilde{t})$, $i = \{1, 3, 4\}$, only two terms are necessary since $0 < \Re(\epsilon)(t_i) < 1$. Strictly speaking truncating the terms as described does not give the expression given by Equation (F.34) since the order of the lowest order of the first terms ignored in the $P_i(\tilde{t})$ is 0.0839. Equation (F.34) should be rewritten as

$$x_i(t) = P_i(\tilde{t}) + \mathcal{O}(\tilde{t}^{1.0839}).$$

The initial conditions given by Equations (6.23), (6.24) and (6.25) may be transformed to the Cauchy initial conditions

$$N_{G1}(0) = 1,$$  \hspace{1cm} (F.42a)

$$\left. \frac{dN_{G1}(t)}{dt} \right|_{t=0} = \frac{39}{20},$$  \hspace{1cm} (F.42b)

$$\left. \frac{d^2N_{G1}(t)}{dt^2} \right|_{t=0} = \frac{2117}{1200},$$  \hspace{1cm} (F.42c)

and

$$\left. \frac{d^3N_{G1}(t)}{dt^3} \right|_{t=0} = \frac{124751}{72000},$$  \hspace{1cm} (F.42d)

which in terms of the variable $\tilde{t}$ become

$$N_{G1}(1) = 1,$$  \hspace{1cm} (F.43a)

$$\left. \frac{dN_{G1}(\tilde{t})}{d\tilde{t}} \right|_{\tilde{t}=1} = \frac{39}{40},$$  \hspace{1cm} (F.43b)

$$\left. \frac{d^2N_{G1}(\tilde{t})}{d\tilde{t}^2} \right|_{\tilde{t}=1} = \frac{2563}{4800},$$  \hspace{1cm} (F.43c)

and

$$\left. \frac{d^3N_{G1}(\tilde{t})}{d\tilde{t}^3} \right|_{\tilde{t}=1} = \frac{485831}{576000},$$  \hspace{1cm} (F.43d)
Solving Equation (F.25), with $x_i(t)$ given by Equation (F.41), for $\tilde{C}_i$ for $i = \in \{1, 2, 3, 4\}$ using the initial conditions given in Equations (F.43) yields

\[
\begin{align*}
\dot{\tilde{C}}_1 &= -1.6754, \\
\dot{\tilde{C}}_2 &= 2.7045, \\
\dot{\tilde{C}}_3 &= -0.0128 + 0.9786i, \\
\dot{\tilde{C}}_4 &= 0.0128 - 0.9786i.
\end{align*}
\]  

A plot of this solution together with the solution obtained using Maple's built-in numerical integrator is shown in Figure F.1. As can be seen the first order approximation given by this technique is in good agreement with Maple’s numerical approximation.

Figure F.1: Comparison of Solutions Obtained Using a First Order Frobenius Technique and Maple’s Numerical Integrator for a Cytotoxic Drug-Cell Cycle Model where Rate Constants Are Not Equal.
References


[42] Maplesoft, Maple 16.01.


Scientific Contribution

Prizes and Awards

- First Prize Awarded for Poster Presentation at the PGR Conference, University of Surrey (2013).

List of Publications


List of Invited Presentations

- A Study of Long Term Mortality from Heart Disease After Radiotherapy for Breast Cancer, Doctoral Training Centre, University of Surrey (2011).
- Modelling Tumour Oxygenation and Tissue Activity Curves for [18F]-Fmiso PET Data, Doctoral Training Centre, University of Surrey (2011).
Modelling and Detecting Tumour Oxygenation Levels

Anne C. Skeldon1*, Gary Chaffey1, David J. B. Lloyd1, Vineet Mohan2, David A. Bradley3, Andrew Nisbet3,4
1 Department of Mathematics, University of Surrey, Guildford, Surrey, United Kingdom, 2 Department of Mathematics, Eidgenössische Technische Hochschule, Zürich, Switzerland, 3 Department of Physics, University of Surrey, Guildford, Surrey, United Kingdom, 4 Department of Medical Physics, Royal Surrey County Hospital, Guildford, Surrey, United Kingdom

Abstract
Tumours that are low in oxygen (hypoxic) tend to be more aggressive and respond less well to treatment. Knowing the spatial distribution of oxygen within a tumour could therefore play an important role in treatment planning, enabling treatment to be targeted in such a way that higher doses of radiation are given to the more radioresistant tissue. Mapping the spatial distribution of oxygen in vivo is difficult. Radioactive tracers that are sensitive to different levels of oxygen are under development and in the early stages of clinical use. The concentration of these tracer chemicals can be detected via positron emission tomography resulting in a time dependent concentration profile known as a tissue activity curve (TAC). Pharmaco-kinetic models have then been used to deduce oxygen concentration from TACs. Some such models have included the fact that the spatial distribution of oxygen is often highly inhomogeneous and some have not. We show that the oxygen distribution has little impact on the form of a TAC; it is only the mean oxygen concentration that matters. This has significant consequences both in terms of the computational power needed, and in the amount of information that can be deduced from TACs.

Introduction
The rapid growth that is frequently associated with malignant tumours results in regions of the tumour becoming low in oxygen, in other words, hypoxic. Understanding tumour hypoxia is important because hypoxic cells are both more aggressive and harder to treat [1,2]. Furthermore, low oxygenation promotes the growth of blood vessels within the tumour (angiogenesis) contributing to the transition from avascular to vascular tumour growth [3]. Yet tissue hypoxia is difficult to identify in vivo. Invasive techniques, such as the use of an Eppendorf probe, only give local information and can seed cancerous cells along the line of entry.

Non-invasive techniques for the detection of oxygen using positron emission tomography (PET) scans are in the early stages of clinical practice. With PET scanners, a patient is first injected with a radioactive isotope of a molecule that takes a prominent part in whatever process is of interest; most radioactive tracers that are in clinical use focus on the metabolisation of glucose but there are some new tracers, such as [F-18]-Fluoromisonidazole (Fmiso) and Cu64 diacetyl-bis(N4-methylthiosemicarbazone) (Cu64-ATSM), that are being developed to detect regions of low oxygen concentration. The tracer is distributed around the body by the blood. In the case of glucose detecting tracers, the highest concentrations of the tracer will occur in very active areas, such as tumours. Similarly with Fmiso or Cu64-ATSM the tracer will accumulate in areas of hypoxic tissue. The PET scanner detects the radiation that is emitted from the tracer as it undergoes radioactive decay, and an image of the concentration of the tracer at different parts of the body can then be re-constructed. This re-construction process is difficult resulting in images of relatively poor resolution, typically 2 –3 mm³. The time dependent decay signal from the PET scanner is known as the tissue activity curve (TAC).

The concentration of the tracer at any location gives a qualitative picture of the degree of tumour hypoxia. Padhani [1] notes that in clinical settings, such qualitative imaging can work well enough, but does introduce a level of subjectivity and that there is a need for greater quantitative understanding. In fact, the concentration of the tracer at any given location is not related to the oxygen concentration of the tissue in a trivial manner and knowing the quantitative relationship between the tracer concentration and tissue oxygenation levels is of great importance if accurate deductions as to the radio-resistance of the tissue are to be made [4]. Indeed, an image created by a snapshot at a single point in time can give a misleading impression because, while for normal tissue the TAC drops after an initial peak, for hypoxic tissue there tends to be a gradual increase in the TAC. This can result in a cross-over point where TACs from both normal and hypoxic tissue give the same result [5] and it is therefore important to consider the TAC at multiple time points. Methods that fit TACs to a nonlinear mathematical model that includes the pharmaco-kinetic behaviour of the tracer and thereby translate the concentration of the tracer to the oxygenation level of the tissue have been developed. The most widely tested of these mathematical models...
have been compartment models [5–8]. These divide the tracer into, typically, three compartments: tracer in the blood plasma; tracer that diffuses freely in the tissue, and tracer that is bound to the tissue via a reaction that is dependent on the concentration of oxygen. The resulting pharmacokinetic (PK) models have defined rates of transfer between the different compartments and results in a set of ordinary differential equations that can be solved analytically. The total TAC is a weighted sum of the signal from each of the compartments. The weights and some of the transfer coefficients are calculated by fitting the experimentally determined TACs to the TACs produced by the PK model. The values of the weights and the transfer coefficients are then used to deduce whether the tissue is hypoxic and what kind of hypoxia occurs. Proof of concept experiments have been carried out which demonstrate that PK models have the ability to qualitatively reproduce the features of TACs and distinguish between different types of hypoxia. However, compartment models take no account of the spatial distribution of oxygen. So for PET scan data fitting can be done for each individual 2mm³ voxel but there is an inherent assumption that the tissue within that voxel is homogeneous and can be represented by an average value. This is not necessarily the case in vascular tumours, the vessels that deliver oxygen tend to be irregular and tortuous making it likely that the distribution of oxygen within a voxel is highly inhomogeneous. There has been some initial work that includes space explicitly by including tracer diffusion in the tissue and allowing the concentration of oxygen to vary from one point to the next, initially by Kelly and Brady [9] and subsequently by Munnich et al [10]. These studies replace the ordinary differential equation compartment model with partial differential equations. Our original interest was in comparing a partial differential equation model for tracer reactions and diffusion with the analogous compartment model to investigate whether the inhomogeneity of the distribution of blood vessels actually matters on the scale of a voxel. However, this comparison is dependent on first establishing the oxygen distribution within the tissue and that has led to a number of other considerations. In general, if more than a qualitative understanding is to be developed, then one needs to be able to quantify the uncertainties/ errors that occur, be they uncertainty that is introduced because of modelling assumptions (for example, whether the tissue can be treated as homogeneous or not), uncertainty due to the difficulty of experimentally measuring parameters that are critical to the model behaviour and finally, computational errors that are introduced due to numerical inaccuracies.

Consequently, the aim of this paper is three-fold. Our first aim is to understand the impact of two particular modelling assumptions. The first relates to the way that oxygen is delivered to tissue. This is a subject that many authors have focussed on and a review article on this subject is given by Goldman [11], yet in even the simplest models of oxygen diffusion and consumption different authors have used different methods and, as we will see, these different assumptions can give quantitatively quite different results. In particular we find that modelling the discrete blood vessels by a ‘source’ term gives a good approximation to the, more realistic, mixed boundary conditions between the vessel walls and the tissue and suggests that efficient algorithms in three-space dimensions could be developed using a source method. The second modelling assumption that we examine is to what extent, on the scale of a voxel, it is important to take account of the spatial distribution of the oxygen in deducing information from TACs, by comparing the results of a partial differential equation model that accounts for oxygen and tracer diffusion with the analogous compartment model.

Our second aim is to examine the sensitivity of the computed oxygenation level of tissue to the various parameters in the model. Measuring physical parameters such as consumption rate of oxygen, diffusivity of oxygen and permeability of blood vessels and the distribution of oxygen is challenging, making it hard to validate any particular mathematical model. However, by understanding the mathematical models one can examine which parameters have a significant effect on predictions that are made by a model and, therefore, which parameters one needs to find for an accurate prediction or, equivalently, to what extent the uncertainty in a particular parameter leads to an uncertainty in the results.

Our final aim is to demonstrate the impact of numerical error that results in the solution of the partial differential equations on too coarse a mesh. We provide computational parameters where the discrete approximations can confidently be considered to be close to the continuous PDE solution.

The paper is outlined as follows. In section 1.1 the simplest models for the diffusion and consumption of oxygen in tissue are re-visited. The different approaches to the different ways of modelling the boundary between the vascular structures that deliver the oxygen and the tissue are examined and a limit is derived in section 1.2 where one expects the mixed boundary conditions used by some authors [12] and the Dirichlet boundary conditions of others [13] to give similar results. In order to separate out the effects of the parameters on the oxygen distribution as compared with how multiple vessels modify the final oxygen distribution we consider a sequence of problems. In section 1.3 we look at just a single vessel and then, we consider a pair of vessels at different distances apart in section 1.4. In section 1.5 we consider multiple vessels and examine to what extent the multivessel results can be understood as a superposition of the single vessel results. In section 2.1 we introduce the particular model of tracer reaction and diffusion that we have studied and examine first the tracer dynamics around a single vessel in section 2.2 and then for multiple vessels in section 2.3, comparing the TACs that results from both random and regular arrangements of vessels. In section 2.4 we fit the TACs produced by the partial differential equation model with a compartment model. In spite of the heterogeneity of the oxygen model we find that the compartment model can distinguish between different levels of oxygen.

### Analysis

#### 1. Oxygen Distribution

Many mathematical models of oxygen transport are built on the Krogh-Erlang cylinder model [14] that models oxygen transport by a diffusive process through a homogeneous medium governed by the equation

$$\frac{\partial P}{\partial t} = D \nabla^2 P - K,$$

(1)

for the oxygen partial pressure $P$ within the tissue, where $D$ is the diffusivity of oxygen in tissue and $K$ describes oxygen consumption by the tissue. In the original Krogh-Erlang model [14], the oxygen partial pressure was fixed at the vessel wall (a Dirichlet boundary condition) with the consumption rate $K$ set to be constant. This latter assumption means that equation (1) has to be supplemented with a requirement that the consumption rate is zero when $P$ is zero to prevent the equations from giving solutions with regions of negative partial pressures. A more realistic form for the oxygen consumption term in equation (1) is the Michaelis-Menten form,
With this nonlinear consumption rate, as $P \to \infty$ the consumption asymptotes to the constant value $K = K_{\text{max}}$, so that when oxygen is abundant, consumption is approximately constant. However, when oxygen is scarce, oxygen consumption is proportional to the amount of oxygen available. This choice for $K$ means that the oxygen partial pressure remains positive (or zero) at all times.

In Goldman [11] all the underlying assumptions of the Krogh-Erlang cylinder model are listed and a thorough review of current work that relaxes these assumptions is given. Of particular relevance here is the intravascular $O_2$ resistance (IVR); in the original Krogh-Erlang model the use of Dirichlet boundary conditions at the vessel wall excluded the possibility that the oxygen delivery to the tissue may be dependent on the partial pressure difference across the vessel wall. As discussed further below, this is only valid if the vessel wall is sufficiently permeable to oxygen.

One way of including IVR is to ignore intravascular processes but to model the flux of oxygen as it diffuses across the vessel wall, and then on into tissue explicitly via a mixed boundary condition, sometimes known as a Robbins boundary condition. This mixed boundary condition arises as follows: assuming that a blood vessel wall consists of two concentric cylinders of outer radius $R$ with width $w$ between the two cylinders, as shown in cross-section in figure 1, and that there is just diffusion and no consumption by the wall tissue, then the flux at $r = R$, $F_R$ is given by

$$F_R = -D_w \frac{\partial P}{\partial n}|_R = -
\frac{D_w}{R} \frac{P_0 - P}{\ln(1 - \frac{w}{R})}$$

(3)

where $D_w$ is the diffusivity in the wall and $P_0$ is the partial pressure of oxygen inside the vessel. For capillaries $w < R$, typical vessel radii are $7 \mu m$ [12] and vessel walls are $0.2 - 1 \mu m$ [9]) and equation (3) becomes

$$F_R \approx \frac{D_w}{w} (P_0 - P) = P_m (P_0 - P),$$

(4)

where $P_m = D_w/w$ is the permeability of the vessel. The inclusion of IVR can therefore be modelled by using the boundary condition (4) at the vessel wall.

An alternative model that includes IVR is to model the vessels by a so-called distributed source where instead of modelling the vessels as discrete entities leading to the solution of the diffusion equation on a punctured domain, the source is represented by a function which has localised spikes at the vessel positions [9]. With such a source term, the diffusion model becomes

$$\frac{\partial P}{\partial t} = D \nabla^2 P - K + \frac{2P_m}{R} (P_0 - P) S.$$

(5)

where $S$ is referred to as the vascular map and is a function that takes the value 1 for regions inside the vessel and 0 otherwise. This is a modification of a term that was first introduced by Baxter and Jain [15] for modelling tumours at the whole tumour scale. The motivation for the particular form for the source term comes from considering the flux across a membrane as in equation (4). Then the net rate of oxygen diffusing for an individual blood vessel per unit volume is given by

$$\frac{1}{\pi R^2 L} \int_{\partial S} F \cdot n = \frac{1}{\pi R^2 L} (2\pi RF_R) = \frac{2P_m}{R} (P_0 - P),$$

(6)

where $\partial S$ is the surface of the blood vessel. So the diffusion model (1) then becomes equation (5).

The derivation of equation (4) and subsequently equation (6) assume that oxygen within the blood vessel is well-mixed and that consequently the partial pressure at the interior of the vessel is fixed at $P_0$. Detailed earlier work by Hellums and co-workers has shown that IVR actually arises as a consequence of the way that oxygen is transported and released by red blood cells [16,17]. Hellums et al [17] showed that the delivery of oxygen to tissue could be described well by a flux of the same form as equation (6), where $P_0$ is the partial pressure in the vessel corresponding to the mean haemoglobin saturation.

Some studies have included IVR [18] and some have not [13], but there has been no systematic comparison of the two. Likewise, although some authors have used source terms [9,12] and some have used models that describe capillaries as discrete entities there has been no comparison of these two methods. This is relevant because, Dirichlet boundary conditions may sometimes be used for the pragmatic reason that they are easier to code but, in fact, can only be justified in the situation that the permeability of the wall is sufficiently high. Similarly, there are computational advantages to having a domain that is simply connected, as occurs if the source term formulation is used. A number of studies have investigated oxygen diffusion in three space dimensions [18,19]. However, the difficulty in correctly implementing the vascular structure and the high computational cost of such simulations mean that it is valuable to thoroughly examine the modelling issues relating to the boundary conditions at the vessel wall in two space-dimensions before considering the three-dimensional problem.

In the rest of this section a quantity that determines whether Dirichlet boundary conditions are appropriate is derived. Then, in order to examine which parameters are significant in determining the level of oxygenation we non-dimensionalise the equations and consider a sequence of problems: first considering the impact of the parameters on the oxygen distribution created by a single vessel and then examining how a pair of vessels interact before, finally, considering tissue with realistic vascular structures.

### 1.1 Mixed versus Dirichlet boundary conditions

If a boundary is sufficiently “leaky”, one would expect mixed and Dirichlet boundary conditions to give the same results. An idea for what is “sufficiently leaky” can be obtained by considering steady-states of equation (1) for a single vessel which satisfy

$$\nabla^2 P = K.$$

(7)
For a cylindrical vessel with no axial dependence, this reduces to
\[
\frac{1}{\mathcal{D}} \frac{d}{dr} \left( \frac{d}{dr} P \right) = \frac{K}{\mathcal{D}}. \tag{8}
\]

In case that \( K \) is constant, equation (8) is exactly soluble and gives
\[
P = \frac{1}{4 \mathcal{D}} r^2 + A \ln r + B,
\]
where \( A \) and \( B \) are integration constants. At some radius \( r = r_m \) the oxygen partial pressure will drop to zero and there will be no flux of oxygen. Applying the boundary conditions \( P = dP/dr = 0 \) at \( r = R \) gives
\[
P = \frac{1}{4 \mathcal{D}} (r_m^2 - R^2) - \frac{1}{2} K r_m^2 \ln \frac{R}{r_m}.
\]

The maximum oxygen diffusion distance, \( r_m \), is determined by the boundary condition at \( r = R \). Using \( P = P_0 \) at \( r = R \) leads to the equation
\[
r_m^2 - R^2 + 2r_m^2 \ln \frac{R}{r_m} + 4DP_0 \frac{K}{\mathcal{D}} = 0.
\tag{9}
\]

Using the mixed boundary condition gives
\[
r_m^2 - R^2 + 2r_m^2 \ln \frac{R}{r_m} + 4DP_0 \frac{K}{\mathcal{D}} = 0.
\tag{10}
\]

As \( \frac{2D}{P_m R} = 0 \) equations (9) and (10) for \( r_m \) become identical, suggesting that provided \( \frac{2D}{P_m R} \ll 1 \) both mixed and Dirichlet boundary conditions will give similar results. Typical values for \( D, P_m \) and \( R \) for tumour blood vessels (see table S1), result in a value for \( \frac{2D}{P_m R} > 1 \), suggesting that Dirichlet boundary conditions are unlikely to give similar results to mixed boundary conditions.

1.2 Non-dimensionalisation. The original problem has six parameters describing tissue and vessel properties, namely, the tissue consumption parameters \( P_{90} \) and \( K_{\text{max}} \), the oxygen diffusivity \( D \), the permeability of the blood vessel to oxygen \( P_m \), the partial pressure of the oxygen within the blood vessel \( P_0 \) and the vessel radius, \( R \). The process of non-dimensionalisation shows that the six tissue and vessel parameters are not truly independent, and the problem can be reduced to just three non-dimensional parameters namely, the scaled partial pressure inside the vessel \( u_0 \); the scaled permeability \( P_m \) and the scaled vessel radius, \( \hat{R} \). The advantage of studying the non-dimensionalised equations is that one has a much reduced parameter space to investigate.

The equations are rescaled by defining \( P = P_{90} u \) and scaling the length by \( \sqrt{DP_{90}/K_{\text{max}}} \). Consequently, for the steady-state solution of the reaction-diffusion equation with rate given by equation (2) three different problems are considered: (i) Dirichlet boundary conditions, (ii) mixed boundary conditions, (iii) distributed source term. These are listed below.

For Dirichlet boundary conditions:
\[
\nabla^2 u = \frac{u}{u+1} \quad \text{for} \quad (x,y) \in (0,L_x) \times (0,L_y), \quad (x,y) \notin \text{vessel}, \quad (11)
\]

\[u(\text{vessel wall}) = u_0.\]

For mixed boundary conditions:
\[
\nabla^2 u = \frac{u}{u+1} \quad \text{for} \quad (x,y) \in (0,L_x) \times (0,L_y), \quad (x,y) \notin \text{vessel} \quad (12)
\]

\[\nabla u |_{\text{vessel wall}} = -\frac{P_m}{R} (u_0 - u),\]

For the source term:
\[
\nabla^2 u = \frac{u}{u+1} - \frac{2P_m}{R} (u_0 - u) S \quad \text{for} \quad (x,y) \in (0,L_x) \times (0,L_y), \quad (13)
\]

where
\[
S = \begin{cases} 1 & \text{for} \ (x,y) \in \text{vessel interior} \\ 0 & \text{for} \ (x,y) \in \text{vessel} \end{cases}
\]

Typical values for the measured physical parameters are listed in table S1 and the corresponding ranges of values for the non-dimensional parameters are given in table S2.

1.3 Computations for a single vessel. With a single vessel the diffusion problem is axi-symmetric and the diffusion problem in two-space dimensions can be reduced to a diffusion problem in one, radial, direction. For example, equation (12) becomes
\[
\frac{1}{\mathcal{D}} \frac{d}{dr} \left( \frac{d}{dr} \right) = \frac{u}{u+1} \quad \text{for} \quad r = \hat{R}, \quad u(\hat{R}) = u_0. \quad (14)
\]

and the source case becomes
\[
\frac{1}{\mathcal{D}} \frac{d}{dr} \left( \frac{d}{dr} \right) = \frac{u}{u+1} - \frac{2P_m}{R} (u_0 - u) S \quad \text{for} \quad r = 0, \quad (15)
\]

where
\[
S = \begin{cases} 1 & r < \hat{R} \\ 0 & r \geq \hat{R}. \end{cases}
\]

Each case leads to a boundary-value problem. For the flux and Dirichlet cases this problem was solved on the large but finite domain, \( \hat{R},\hat{L} \), corresponding to the region outside a vessel of radius \( \hat{R} \). The value for \( L \) was chosen sufficiently large (typically \( L = 20 \)) that the results were independent of whether Dirichlet or Neumann boundary conditions were chosen at \( r = L \). In the cases of the source term, \( \hat{u}(0,\hat{L}) \) with \( \partial \hat{u}/\partial r = 0 \) at the lefthand boundary. Each one-dimensional problem was solved using the matlab boundary value problem solver bvp4c; typical solutions are shown in figure 2. All boundary conditions lead to a similar,
monotonically decreasing profile: in fact the maximum principle can be used to show that the maximum value of the oxygen has to occur on the boundary. The difference between the various boundary conditions is that with Dirichlet boundary conditions, this maximum value is pinned to the value of $u_0$, the scaled partial pressure in the blood vessel, but in all other cases the maximum value is at some value that is lower than this. The consequence of this pinning of the partial pressure to the value of $u_0$ at the vessel boundary is that the Dirichlet boundary conditions tend to give higher levels of oxygenation than mixed boundary conditions or the source term. On figure 2 the vertical dashed line represents the boundary of the vessel. The ‘maximum diffusion distance’ for oxygen in tissue is often quoted as 70 microns, equating to a distance below which the level of oxygen is too small to be detected.

In order to compare the different solutions systematically as the parameters are varied, we have considered two different measures. Firstly the the L1 norm, $||u||_{1}$, where

$$||u||_{1} = \int |u| dx \; dy.$$  \hspace{1cm} (16)

The L1 norm is related to the average level of oxygenation, $\bar{u}$, of a piece of tissue of area $A$ by

$$\bar{u} = \frac{||u||_{1}}{A}.$$ 

Oxygenation of tissue samples on the microscale are often examined using tissue staining where a dye that is oxygen sensitive is applied to a tissue slice. This tends to lead to a binary measure: either oxygen is present or not (or only at a concentration below a threshold value). Results are often quoted as a hypoxic fraction, that is the fraction of the tissue that is hypoxic. In order to mimic this kind of measure we have also calculated the ‘oxygenated area’, $A_{ox}$, which is the area for which the oxygen partial pressure is greater than a threshold value $u_0$.

For a given area of tissue $A$ the hypoxic fraction $A_h$ could then be calculated from

$$A_h = \frac{A - A_{ox}}{A}.$$  \hspace{1cm} (17)

As can be seen from figure 2, the calculated value for the oxygenated area will depend strongly on the particular value of the threshold $u_0$ that is chosen: for the Dirichlet boundary case depicted in figure 2, threshold values $u_0 = 4$ and $u_0 = 2$ lead to oxygenated radii of 2.28 and 3.15 respectively. In turn, these values lead to oxygenated areas of 16.3 for $u_0 = 4$ and nearly double that value, 31.2 for $u_0 = 2$. Different threshold values are important for different aspects of a cell function, but broadly oxygen levels below 5—15 mmHg ($u_0 = 2—6$, for typical parameter values) have a significant impact on the outcome of cancer therapies [1]. In the case of radiation treatment, half-maximal sensitivity to radiation therapy occurs at oxygen levels of 2—5 mmHg ($u_0 = 0.8—2$). Typical tissue staining techniques stain tissue at threshold values of between 5 mmHg and 10 mmHg ($u_0 = 2$ and 4). Given the sensitivity of the results to the value of the threshold, if comparison is to be made with experimental data, it is particularly important that an accurate value for this threshold is known and this in itself can be difficult. In Pogue et al [12] a careful study fitting a diffusion model for oxygen with vascular maps derived from real tissue samples was performed. They found their results were very sensitive to the threshold that was chosen and that their model fitted the data best for a value for the threshold that was much lower than the conventionally accepted value. For many of the results that are presented in this paper, we have selected the value $u_0 = 2$.

Results for the oxygenated area for fixed radius but varying vessel partial pressure $u_0$ and permeability $P_m$ are shown in figure 3. As is to be expected, these show that at high permeability, all three sets of boundary conditions give similar results. At low permeability, the source term representation gives a reasonable approximation to the mixed boundary conditions. Note that the condition found in §7 for the Dirichlet and flux boundary conditions to coincide translates to $P_m R \gg 2$ or, for $R = 0.55$, $P_m \gg 3.6$. For the experimentally measured range of values of $P_m = 0.55—2.75$, figure 3(c) and (d) show that modelling oxygenation using Dirichlet rather than flux boundary conditions will result in an over estimate for the oxygenated area and that this is more significant the lower the vessel partial pressure. So, for example, for the low scaled vessel partial pressure of $u_0 = 8$ (equivalent to 20 mmHg), mixed boundary conditions give an oxygenated area of 4.9 and Dirichlet boundary conditions give a value that is more than two and a half times bigger of 12.7. Even at the highest scaled vessel partial pressures, $u_0 = 40$ (equivalent to 100 mmHg), mixed boundary conditions give an oxygenated area of 57.0 and Dirichlet boundary conditions give a 50% larger value of 86.1.

In figure 4 the oxygenated area for varying scaled vessel radius is shown. This show that the oxygenated area is approximately linearly related to the scaled vessel radius. Together, figures 3 and 4 show that, for the typical ranges of permeability that are quoted for blood vessels, it is important to take account of the IVR and that either using a mixed boundary
condition or a source term will give similar results. The oxygenated area is sensitive to the scaled vessel partial pressure and to the scaled permeability and an uncertainty of 10% in either of these values will lead to a similar order of uncertainty in the oxygenated area. The oxygenated area is much less sensitive to the scaled vessel radius.

1.4 Two vessels.

In a piece of tissue there is typically many vessels, not just a single isolated one. If two vessels are sufficiently far apart, then each will be unaffected by the presence of the other, as illustrated in figure 5(a) and (c). As they become closer and closer, the oxygen distribution around each vessel will become more and more affected by its neighbour, see figure 5(b) and (d).

The computations shown in figure 5 were carried out on a two-dimensional domain with a grid of $401 \times 201$ grid points using a square mesh of grid size $0.1$. Grid refinement checks were carried out to check that this was sufficiently fine (see table 1). The grid refinement checks suggest that, for results that are accurate to 10%, a grid of between two and four grid points per vessel should be used. We note that in order to minimize computational cost, previous studies have frequently used a grid of spacing of the same size as the vessel and that this will introduce an error of $30 - 40\%$, depending on the type of boundary conditions used.

We have systematically examined how the $L^1$ norm and the oxygenated area vary as the separation between the vessels is changed and the results are summarised in figure 6 as a function of the separation. Only the oxygenated area is shown as the results for the $L^1$ norm are qualitatively similar. For vessels sufficiently far apart, the $L^1$ norm and the oxygenated area are twice the values calculated in §2 for one vessel. This corresponds to the flat section to the far right of figure 6 and shows that for a separation $s$ greater than about ten the vessels interact only minimally. Note that in these non-dimensional units, this represents a separation of approximately nine vessel diameters.

As the vessels get closer, the oxygenation of the tissue initially increases but then decreases approaching the value of the oxygenation produced by a single vessel as $s \to 0$. The increase is much more prominent in the $L^1$ norm than in the oxygenated area, reflecting the fact that the dominant effect of two vessels close together is that the level of oxygenation of the oxygenated tissue increases, rather than that more tissue reaches the oxygen threshold value of $u_0$.

1.5 Multi-vessel.

In normal tissue, blood vessels are regularly spaced in order to deliver oxygen to tissue in an optimal manner. In tumour tissue, this is not the case and the position of blood vessels is much more closely represented by a random distribution, resulting in a log-normal distribution for the distance between blood vessels.

First we outline how we distribute vessels on the plane while still being able to carry out computational grid refinements. In order to randomly place the vessels without overlap we first construct a ‘vascular grid’ that has a grid length of $2R$. Vessels are placed so that their centres are at random points of the vascular grid. The choice of grid length means that no vessels can overlap each other. A computational mesh is then constructed that forms a sub-grid of the larger vascular grid, one example is shown in figure 7. This computational mesh can be refined to give adequate numerical
Computations were carried out on a domain of $55 \times 55$ in non-dimensional units, equating to $1mm^2$ of tissue for typical values of the parameters. As for the two vessel case, a grid spacing of $0.1$ gave good resolution. The effect of varying microvessel density (MVD), was considered by solving equations (13) for a sequence of different MVDs. For each value of the MVD, ten different random vascular maps were created and the $L^1$ norm and the oxygenated area calculated. The random selection of

Figure 4. Oxygenated area of a single vessel for $u_0 = 2$ and varying vessel radius, $\bar{R}$. (a) $P_m = 2, \mu_0 = 8$. (b) $P_m = 2, \mu_0 = 40$. (c) $P_m = 50, \mu_0 = 8$. (d) $P_m = 50, \mu_0 = 40$. doi:10.1371/journal.pone.0038597.g004

resolution. Computations were carried out on a domain of $55 \times 55$ in non-dimensional units, equating to $1mm^2$ of tissue for typical values of the parameters. As for the two vessel case, a grid spacing of $0.1$ gave good resolution. The effect of varying microvessel density (MVD), was considered by solving equations (13) for a sequence of different MVDs. For each value of the MVD, ten different random vascular maps were created and the $L^1$ norm and the oxygenated area calculated. The random selection of

Figure 5. Contour plots and solution profiles for two vessels placed at different separations. In all cases, $u_0 = 16, P_m = 2.75, \bar{R} = 0.55$ and the source model for the delivery of oxygen to the tissue was used. (a) Contour plot for two widely separated vessels, separation $s = 15$. (b) Contour plot for two vessels that are close enough to interact, separation $s = 5$. (c) Oxygen concentration profile along the line $y = 0$ for $s = 15$. (d) Oxygen concentration profile along the line $y = 0$ for $s = 5$. doi:10.1371/journal.pone.0038597.g005
points on the vascular grid results in vascular maps which have a log normal distribution of nearest neighbour distances. As the MVD increases, both the mean and the variance of this distribution decreases (mean \(\propto \sqrt{MVD}\), variance \(\propto \frac{1}{MVD}\)). We have considered MVDs in the range \(0-200\) per mm\(^2\), which includes the values used in previous studies of tumour oxygenation [9,13].

Commonly quoted values for vessel partial pressures range from 20mmHg to 100mmHg where, 100mmHg is typical of arterioles and 40mmHg typical of venules. Often tumour supply is from the venule side, and although some of the results that are presented below are for the full range from 20mmHg to 100mmHg (8 to 40 in nondimensional units), more detailed results are shown for the case of vessel partial pressure 40mmHg (16 in nondimensional units). The results for the fraction of the area of the tissue that is oxygenated for three different values of the vessel partial pressure \(u_0\) and varying hypoxic levels \(u_h\) are shown in figure 8. The general trends are not surprising: more vessels are needed to oxygenate more tissue up to some cut-off number beyond which all the tissue is oxygenated; the vessel density that is needed for the tissue to be oxygenated depends on the value that is used to specify oxygenation (\(u_0\)), with more vessels needed the higher the value of \(u_0\). Typically, tissue is considered to be hypoxic if it has partial pressures less than 1mmHg. So, for example, in figure 8(b) corresponding to vessel partial pressures of \(u_0 = 16\) (40mmHg) and for a micro-vessel density of 50 vessels/mm\(^2\), typically 90% of the tissue receives some level of oxygen, but for most of the tissue this is at too low a level to be significant resulting in the fact that only approximately 15% is oxygenated, 35% is hypoxic and the remaining 50% is necrotic.

The computational cost of simulating multi-vessel distributions to attain average quantities leads one to ask whether one could predict the multi-vessel results just from the one-vessel results. In particular, if the vessel density is low, one might expect the oxygenated area of the multi-vessel distribution to simply be the oxygenated area given by a single vessel multiplied by the number of vessels, i.e.

\[ A_{ox} \approx N A_{ox}, \]

where \(N\) is the number of vessels and \(A_{ox}\) is the oxygenated area of a single vessel. Figure 9 shows the oxygenated area for two different values of \(u_0\) in more detail and compares the results with a number of approximations. We focus on the value of scaled vessel partial pressure \(u_0 = 16\) (\(P_0 = 40\)mmHg for typical parameter values) since this is the most widely quoted value for the vessel partial pressure in tumour tissue. For microvessel densities up to around 50 mm\(^{-2}\), the vessels do not interact, and the approximation give by equation (18), the dashed line in the figure, works well. That the vessels do not interact is further underlined by the fact that up to a MVD of around 50, there is no difference in the oxygenated area produced by a regular grid of vessels (shown by the thick line in figure 9) and that produced by a random arrangement of vessels.

2. Tracer

Having discussed some aspects of modelling oxygen diffusion in tissue, we now consider the issues with trying to detect that oxygen using a tracer. First we solve a model for tracer reaction and diffusion that includes the spatial distribution of oxygen. We then examine to what extent a compartment model for tracer dynamics can accurately determine the level of oxygenation.

2.1 Modelling and non-dimensionalisation. The detection of oxygenation via PET scanning techniques first requires a radioactive tracer to be injected into the blood. The tracer is transported by the blood: some is removed from the blood by the kidneys and some diffuses into other body tissues. Once in the tissue, some of the tracer will bind at a rate that is dependent on the local oxygen concentration with the tracer being bound more

\[ A_{ox} \approx NA_{ox}, \]

Source: 10.1371/journal.pone.0038597.g006

<table>
<thead>
<tr>
<th>Table 1. Grid refinement check.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grid spacing</td>
</tr>
<tr>
<td>-----------------------------</td>
</tr>
<tr>
<td>1.0000</td>
</tr>
<tr>
<td>0.5000</td>
</tr>
<tr>
<td>0.2500</td>
</tr>
<tr>
<td>0.1250</td>
</tr>
<tr>
<td>0.0625</td>
</tr>
<tr>
<td>0.0400</td>
</tr>
</tbody>
</table>

The \(L^1\) norm as a function of grid size for two vessels separated by 5 units with \(u_0 = 40\) and \(P_s = 2.75\).
effectively at low oxygen levels. A detailed pharmacokinetic study of the binding process for FMISO was carried out by Casciari et al [6]. Although their compartment model did not allow for any spatial variation, nevertheless they were able to show that the model could replicate typical behaviour of TACs from both a more complicated, but still spatially homogeneous, model and clinically extracted TACs. They did find that including some transport limitations into the compartments representing tracer in the tissue was important.

In order to take account of the diffusion of tracer and the spatial distribution of oxygen, Kelly and Brady [9] suggest taking the model for the partial pressure of oxygen, equation (5) and coupling it to a partial differential equation for the tracer,

\[
\frac{\partial T_f}{\partial t} = D T \nabla^2 T_f - K_{tracer} T_f + \frac{2P_T}{R} (T_{blood} - T_f) \cdot S,
\]

\[
\frac{\partial T_b}{\partial t} = K_{tracer} T_f,
\]  

where \(T_f\) is tracer that is free to diffuse and \(T_b\) is bound tracer. The parameter \(K_{tracer}\) is the rate at which the free tracer is bound and is dependent on the oxygen partial pressure \(P_o\).

The parameter \(K_{tracer}\) and is dependent on the oxygen partial pressure

\[
K_{tracer} = \left( \frac{k_{max} P_o}{P + P_0} \right) \left( \frac{P}{P + P_2} \right).
\]

The second term in brackets acts as a switch to turn off the binding if tissue is necrotic. The concentration of tracer in the blood, also known as the plasma input function, \(T_{blood}\) is modelled as the sum of two exponential decays,

\[
T_{blood} = A (e^{-k_1 t} + be^{-k_2 t}).
\]

The first term represents the dispersal of the tracer around the body, the second the renal term representing the removal of tracer by the kidneys. Typically, \(k_1 < k_2\). Implicit in modelling the tracer in the blood in this way is that the tumour is a small volume compared with the volume of the rest of the body. Consequently the fact that some tracer flows into the tumour has a negligible impact on \(T_{blood}\).

Mönch et al use a similar model [10], but instead of using a source term to model the entry of tracer from the blood they use mixed boundary conditions. In section 1 we found that for oxygen diffusion using a source term gave comparable results to the use of mixed boundary conditions, and we would expect this to be the same for the tracer. Kelly and Brady [9] show that this model can reproduce typical TACs by considering random distributions of vessels. Mönch et al do a similar comparison, but this time using vascular maps that are obtained from tissue staining. Comparing with experimentally determined TACs shows that the partial differential equation model does mimic the behaviour that is seen in practice. Our aim here is to examine to what extent the partial differential equation (19) model is needed in order to accurately model the behaviour that is seen and to what extent a compartment model is adequate.

As for the oxygen problem, we first non-dimensionalise by rescaling space by \(\sqrt{DP_{0}}/K_{max}\) and the oxygen partial pressure \(P = P_{out}\) and introducing \(T_f = A_T T_b = A_{vb}, T_{blood} = A_{vb}, t = D T_{bio}^{-1}\). This results in the model for the oxygen and tracer as:

\[
\frac{\partial A_{vb}}{\partial t} = \frac{\partial^2 A_{vb}}{\partial x^2} - u_{vb} \frac{\partial A_{vb}}{\partial x} - \frac{A_{vb}}{A_T}.
\]

Figure 8. Mean of ten realisations of the oxygenated area versus vessel density (MVD). (a) \(u_{vb} = 4.0\), (b) \(u_{vb} = 2.0\), (c) \(u_{vb} = 0.8\), (d) \(u_{vb} = 0.4\). In all cases, \(P_{out} = 2.75\) and the source model for oxygen entry from the blood vessels is used. The different lines on each plot represent different values of the threshold, \(u_b\) used to measure the level of oxygenation.

doi:10.1371/journal.pone.0038597.g008
\[
\psi u = \frac{u}{u+1} \frac{2p_m}{R} (u_0-u) \cdot S \quad \text{for} \quad (x,y) \in (0,L_x) \times (0,L_y), \quad (21)
\]

and for free and bound tracer,

\[
\begin{align*}
\frac{\partial \psi_f}{\partial t} &= \nabla^2 \psi_f - \left( \frac{kP}{u+P_1} \right) \frac{u}{u+P_2} \psi_f + \frac{2P_T}{R} \left( \psi_{\text{blood}} - \psi_f \right) \cdot S, \\
\frac{\partial \psi_b}{\partial t} &= \left( \frac{k}{u+P_1} \right) \frac{u}{u+P_2} \psi_f, \quad (22)
\end{align*}
\]

where the scaled plasma input function is

\[
\psi_{\text{blood}} = \left( e^{-k_0' t} + be^{-k_1 t} \right), \quad (23)
\]

and

\[
S = \begin{cases} 
1 & \text{for} \quad (x,y) \in \text{vessel interior} \\
0 & \text{for} \quad (x,y) \in \text{vessel}.
\end{cases}
\]

Since at the start there is no tracer in the tissue, only in the blood plasma, both \( \psi_f \) and \( \psi_b \) are set to zero initially.

The scaled parameters for the tracer dynamics are

\[
\hat{P}_T = P_T \sqrt{\frac{P_{50} D_T}{K_{\text{max}}}} \cdot \hat{k}_b = \frac{k_{\text{max}} DP_{50}}{D_T K_{\text{max}}} \cdot \hat{P}_1 = \frac{P_1}{P_{50}}, \hat{P}_2 = \frac{P_2}{P_{50}}, \hat{k}_0 = \frac{DP_{50}}{D_T K_{\text{max}}} \cdot \hat{k}_b. \]

2.2 Single vessel. Before considering how randomly distributed vessels within a piece of tissue behave, we first examine a single vessel. Equations (21) are solved to find the steady-state oxygen distribution as shown in figure 10(a). Then, equations (22) are solved to give the concentrations of free and bound tracer as a function of time and space. For these computations and for those in the following sections we have chosen representative values from the literature for the various parameter values. The parameter ranges are given in table S1 for the dimensioned parameters and in table S2 for the corresponding non-dimensional parameters.

Typical concentrations of the free and bound tracer as are shown in 10(b) and (c) respectively for three different points in time. These show how, initially, the dominant effect is the diffusion of the tracer into the tissue—at \( \tau = 10 \) there is essentially no bound tracer but tracer has diffused a considerable distance from the vessel (note the vessel radius is 0.55 in these non-dimensional units). However, as time goes on the binding process becomes important–by \( \tau = 500 \) the way that the binding is dependent on the oxygen level is apparent, with both the low binding rate at high oxygen concentrations and the effect of the necrotic term resulting in the shape of the bound oxygen profile in 10(c). In fact, the maximal binding rate occurs when \( u = \sqrt{P_1 P_2} \), which for the parameters we have used is \( u \approx 0.38 \).

In order to further illustrate the behaviour, in figure 11(a) the decay of the plasma input function, equation (23), as a function of time is shown and, in figure 11(b) and (c), the concentration of free and bound tracer respectively are shown for three different points.
in space. For the parameters we have chosen the initial concentration of the plasma in the blood vessel is $1.5$. However as $k_0 \ll k_3$ this value rapidly drops, so fast that on the timescale shown this is not visible, and in fact the blood plasma term is dominated by the second term in equation (23). As time continues, the free tracer diffuses from the blood vessels into the tissue, so that at any particular location the free tracer concentration initially increases with time, as seen in figure 11(b). The further from the blood vessel, the longer it takes the tracer to diffuse, so the slower this increase. At the same time as diffusing in space, the free tracer binds at a rate dependent on the oxygen, and this ultimately leads to the decay of the free tracer over time. In figure 11 (c) the growth of the bound tracer as a function of time for three different points are shown. At $r=10$, the bound tracer is zero because the oxygen concentration is so low that the tissue is necrotic. A TAC consists of the sum of signals from the plasma, free and bound tracer,

$$\text{TAC} = \int \left( v_{\text{blood}} + (v_f + v_b)(1-S) \right) dxdy. \quad (24)$$

The general characteristics of TACs from normoxic, hypoxic and necrotic tissue can be seen by considering the three points $r=1$, $r=5$ and $r=10$ respectively, as shown in figure 11. For $r=1$, the sum of the free and bound tracer will show a very rapid increase from zero initially to a high level and then a slower but still fairly rapid decline. Whereas, for $r=10$, the tissue is necrotic and there is effectively no bound tracer. The only tracer contribution to the TAC then comes from the free component, which because of the distance of this point from the blood vessel, shows only a gradual increase that is diffusion limited. The point $r=5$ sits between these two extremes. That there is a cross-over point, as mentioned by Wang et al [5], where both oxygenated and hypoxic tissue would give the same image is clearly seen.

### 2.3 Multi-vessels.

In section 1 it was seen that the distribution of oxygen can be considered as a superposition of the oxygen distribution from single vessels for low enough vessel densities, or equivalently that the oxygen distribution from a regular grid of vessels and that for a random arrangement of vessels give similar oxygenation levels below a vessel density of about 50 for a scaled partial pressure of $u_0=16$ (equating to a partial pressure of 40mmHg if typical parameter values are used). Below, the analogous behaviour is considered for the TAC that results from different microvessel density distributions. For each microvessel density both random and regular vessel distributions are considered. Having specified an arrangement of vessels, the oxygen map is first calculated by solving equations (21). One example of the resulting oxygen map is shown in figure 12 (a). The tracer equations (22) are then solved as a function of time with the plasma input function shown in figure 12(b). The resulting maps for the free and bound tracer at a sequence of points in time are shown in figure 12 (c),(e),(g) and (d),(f),(h) respectively. In figure 12(b) and (c) it is seen how the initial phase is diffusion dominated, with tracer only occurring close to the blood vessels and the bound tracer at a rather lower level than the free tracer. Over time, as seen in (d) and (e) and then in (f) and (g) the free tracer continues to diffuse and is also gradually converted to bound

---

**Figure 11. Plasma input function and tracer time evolution.** (a) Plasma input function. (b) Free tracer against time for three different points in space. (c) Bound tracer against time for three different points in space. (d) Tissue activity curve.

doi:10.1371/journal.pone.0038597.g011
tracer, with highest levels of bound tracer occurring in the hypoxic 'rings' that form around blood vessels. These rings have a maximum value at a distance of around five non-dimensional units and are the two-dimensional manifestation of the maximum seen in the tracer profile in figure 10(c). The corresponding TAC for this square of tissue was then calculated by combining the plasma input function, and the free and bound tracer using equation 24. The resulting TACs for different vessel densities are shown in figure 13. At the lowest vessel density of five vessels per mm$^2$, as shown in the top row, a regular arrangement of vessels would be indistinguishable from a random arrangement of vessels. More surprisingly, even at high vessel densities, the differences between the random arrangement of vessels and the regular arrangements are rather subtle. This suggests that it is in fact not the random nature of the vessel distribution that is most critical, on the scale of a millimetre.

2.4 Comparison with compartment models. Having computed the oxygen map and the resulting TAC, one can ask

---

**Figure 12. Oxygen map and contours of free and bound tracer as a function of time.** A microvessel density of 100 mm$^{-2}$ has been used. (a) Oxygen concentration. (b) Plasma input function as a function of time. (c) $v_f$ at $\tau = 10$. (d) $v_b$ at $\tau = 10$. (e) $v_f$ at $\tau = 50$. (f) $v_b$ at $\tau = 50$. (g) $v_f$ at $\tau = 500$. (h) $v_b$ at $\tau = 500$.

doi:10.1371/journal.pone.0038597.g012
to what extent a compartment model can extract parameters such as the mean level of oxygenation. Previous authors have compared both compartment models and partial differential equation models with real experimental data. The advantage of trying to fit a compartment model with ‘experimental data’ generated from a partial differential equation is that one has much greater knowledge and control over the exact parameter values that are used. If fitting cannot work in this idealised scenario, then it has little hope in the real world.

In order to compare the behaviour of compartment models with a model that includes diffusion of the tracer and the spatial dependence of the oxygen within the tissue we consider the compartment model constructed by Thorwarth et al [7] and used in [5,8,10]. This model considers three compartments, one for the tracer in the blood, one for the free tracer and one for the bound tracer. The tracer in the blood is modelled by equation (20), the remaining two compartments are modelled by the coupled ordinary differential equations,

\[
\frac{dC_f}{dt} = k_1 C_{\text{blood}} - (k_2 + k_3) C_f,
\]

Figure 13. Mean levels of free and bound tracer and TACs computed from the PDE model. Each row corresponds to a different microvessel density (5, 50, 100 and 150 per mm² respectively). The first column shows the contribution to the TAC from the tracer in the blood plasma and the free and bound tracer in the tissue. Ten different random vessel distributions were considered, so ten different sets of curves are shown for each contribution. The central column shows the TACs that result from the ten different random vessel arrangements (solid line) and the TAC as computed from a regular arrangement of vessels (dashed line). The final column shows the hypoxic fraction for each of the different random realisations.

doi:10.1371/journal.pone.0038597.g013
\[ \frac{dC_b}{dt} = k_3 C_f. \quad (25) \]

Here, \( C_f \) represents the free tracer, \( C_b \) represents the bound tracer. The constants \( k_1 \) and \( k_2 \) represent the rate at which tracer enters/leaves the free compartment and is related to the permeability of the vessels to the tracer. The constant \( k_3 \) is the net binding rate of the tracer in the tissue and is related to the level of oxygenation of the tissue. Non-dimensionalising by letting \( v_{\text{blood}} = \frac{AT_{\text{blood}}}{A} \), \( w_f = A C_f \), \( w_b = A C_b \) and \( t = \frac{D H}{D t q} \) leads to the equations

\[ \frac{dv_{\text{blood}}}{dt} = \tilde{k}_1 v_{\text{blood}} - (\tilde{k}_2 + \tilde{k}_1) w_f, \quad (26) \]

\[ \frac{dw_f}{dt} = \tilde{k}_3 v_{\text{blood}} - (\tilde{k}_2 + \tilde{k}_3) w_f; \quad \frac{dw_b}{dt} = \tilde{k}_4 w_f. \quad (27) \]

Initially there is no free or bound tracer so that \( w_f(0) = w_b(0) = 0 \), leading to the analytical solution to equations (27)

\[ \begin{align*}
w_f &= \frac{\tilde{k}_1}{\tilde{k}_2 + \tilde{k}_3 - \tilde{k}_0} e^{-k_0 t} + \frac{\tilde{k}_1 b}{\tilde{k}_2 + \tilde{k}_3 - \tilde{k}_b} e^{-k_b t} \\
- \tilde{k}_3 &\left(\frac{1}{\tilde{k}_2 + \tilde{k}_3 - \tilde{k}_0} + \frac{b}{\tilde{k}_2 + \tilde{k}_3 - \tilde{k}_b}\right) e^{-(\tilde{k}_2 + \tilde{k}_3) t}.
\end{align*} \quad (28) \]

Typical time tracers of \( v_{\text{blood}}, w_f \) and \( w_b \) are shown in figure 14. There are three time scales that are important here corresponding to the three different rates that appear in the exponential terms. Typically \( k_2 \ll k_0 \) and \( k_3 \ll k_2 + k_3 \), but \( k_2 + k_3 \) can be either greater or less than \( k_0 \) depending on the oxygenation level of the tissue. It is the two timescales \( k_4 \) and \( k_3 \) that are relevant for \( v_{\text{blood}} \), and the fact that \( k_3 \ll k_0 \) is seen by the very rapid decline in \( v_{\text{blood}} \) in the first few time units followed by a much slower decline thereafter. For the free tracer, although all three timescales appear in the solution, it is the influence of \( k_2 \) and \( k_3 + k_4 \) that are most clearly seen in figure 14. The concentration of free tracer first increases then decreases over time as tracer first diffuses from the blood into the free compartment and then leaves to become bound. However, the position and height of the consequent maximum in the free tracer depends on how fast the free tracer is converted to bound tracer relative to the dispersion of tracer around the body as is shown by the two cases in figure 14. In 14(b) \( k_2 + k_3 > k_0 \), and as soon as the tracer enters the free compartment it is converted to bound tracer so the amount of free tracer remains low.

The TAC consists of a signal with different weightings of the three components, \( v_{\text{blood}}, w_f \) and \( w_b \). Fitting of the weights along with the rate constant \( \tilde{k}_3 \) are used to give some idea if tissue is hypoxic or not: hypoxic tissue should have a relatively high value for \( \tilde{k}_3 \) and more bound tracer than normal tissue.

**Figure 14. Plasma input function and free and bound tracer concentrations computed from the compartment model.** The parameters \( k_0 = 5, k_1 = 0.1, k_2 = 0.5, k_3 = k_4, b = 0.5 \) are used in both cases and in (a) \( k_2 + k_1 = 0.1 \), in (b) \( k_2 + k_1 = 4 \).

doi:10.1371/journal.pone.0038597.g014
In each case, we first compute a TAC by solving the partial differential equation model for a particular microvessel density. This computed TAC is then fitted to the formula for the TAC given by (33). We assume that the plasma input parameters, $k_0$, and $b$, are known and fit for $k_1$, $k_2$, $k_3$ and the weights $a$ and $b$. A sequence of calculations for increasing microvessel density was carried out, for each of three vessel partial pressures $u_0 = 8$, $u_0 = 16$, and $u_0 = 40$ respectively. The results are summarised in figure 15 and figure 16. The parameters $k_1$ and $k_2$ in the compartment model are the rates at which tracer enters and leaves the free tracer compartment. As can be seen from figure 15, the values of this parameter are dependent on both the vessel partial pressure and the mean oxygenation level—or equivalently the microvessel density. The parameter $k_3$, as shown in figure 15(c), is the rate at which oxygen binds to the tissue, and here the nonlinear relationship between the amount of oxygen and the mean value of oxygen is apparent with a binding rate that is highest for hypoxic tissue and low both for very low levels of oxygen, where tissue is predominantly necrotic, and low for high values of oxygen. The parameters $k_1$, $k_2$, and $k_3$ are strongly correlated with each other, as demonstrated in figure 16. Consequently, without knowing the vessel partial pressure it is not possible to deduce information about the mean oxygen levels or, equivalently, the microvesSEL density from these parameters alone. Values for the parameter $k_3$ do give a clear indication of hypoxia, with the maximum value of $k_3$ occuring for a non-dimensional mean oxygen level of around 1 (corresponding to 2.5 mmHg). Low values of $k_3$ can occur for one of two reasons, either because tissue is necrotic or because tissue is normoxic. The difference between these two cases can be deduced by considering both $k_3$ and $k_1$: normoxic tissue would have a low value of $k_3$ and a high value of $k_1$ whereas necrotic tissue would have a low value of $k_3$ and a low value of $k_1$.

![Figure 15](https://example.com/figure15.png)

**Figure 15. Fitted parameter values.** The fitted values of (a) $k_1$, (b) $k_2$, (c) $k_3$, and (d) $b$ as a function of the mean oxygen level of the tissue. For the computation of each point, first the MVD is fixed. The oxygen map is then computed from equation (21) and the mean oxygen level of the tissue determined. The TAC from the PDE is then constructed by solving equations (22) and computing the expression given in (24). Finally, values of $k_1$, $k_2$, $k_3$, and $b$ are determined by fitting the TAC from the PDE to the compartment model TAC, equation (33). The circles, points and crosses are calculations for different vessel partial pressures: circles represent calculations with $u_0 = 8$, points represent calculations with $u_0 = 16$, and crosses represent calculations with $u_0 = 40$. doi:10.1371/journal.pone.0038597.g015
The parameter $k_3$ in the compartment model represents the binding rate. This rate is dependent on the mean oxygenation level in the free tracer compartment and should be directly related to the binding rate in the partial differential equation model given in equation (22),

$$ k_3 = \left( \frac{k}{u + P_1} \right) \left( \frac{u}{u + P_2} \right). $$

By assuming that the parameters $k, P_1$ and $P_2$ are known, one can invert this relationship and examine to what extent the value of $k_3$ given by fitting the compartment model correlates to the actual mean value of oxygen given by the partial differential equation calculation, see figure 17.

**Discussion**

Modelling the distribution of tracer in the body is a difficult task. There are a number of different levels of uncertainty and inaccuracy. Firstly, in writing down a mathematical model various modelling assumptions are made as to which processes may be neglected and which cannot. Secondly, in most models there are parameters which have to be determined. The value of these parameters can affect both the qualitative and quantitative behaviour of the model. Finally, there are computational errors that are introduced when numerical methods are used to solve equations. If a mathematical model is to be of use, these different types of error need to be quantified and, ideally, an estimate of the uncertainty of any result made.

In this paper we have sought to quantify the effect of some of these sorts of error for the particular problem of oxygen diffusing in a (two-dimensional) piece of tissue and the consequent tracer dynamics. We have addressed two particular modelling issues: firstly the consequence of using different kinds of boundary condition to describe the flow of oxygen from blood vessel to tissue and secondly the extent to which compartment models can be used to describe tracer concentration in tissue where the oxygen distribution is inhomogeneous. For typical vessel permeabilities and partial pressures for tumour tissue, we have found that using a Dirichlet type boundary would typically result in an overestimate of the amount of oxygen by a factor of two, suggesting that either mixed, or the source method should be used. The fact that the source method gives good results, is significant as this is a method that is likely to be easier to implement in three space dimensions than modelling blood vessels as discrete entities with flux boundary conditions. The second modelling assumption that we have investigated is to what extent the heterogeneous nature of the vascular supply is important/detectable by a TAC that averages over a region of a square millimetre. In fact, the actual distribution of the vessels does not significantly affect the form of the TAC: TACs from both regular and randomly arranged blood vessels

![Figure 16. Correlation of $k_1$ with (a) $k_2$, (b) $k_3$, and (c) $\beta$. The circles are computations with a vessel partial pressure $u_0 = 8$; the points are for $u_0 = 16$, and the crosses are for $u_0 = 40$. doi:10.1371/journal.pone.0038597.g016](image)

![Figure 17. Predictions of the compartment model. (a) The value of the mean oxygen level as predicted by fitting the compartment model to the TAC that is computed from the partial differential equation versus the actual mean oxygen level. (b) The predicted value of the parameter $\beta$ versus the actual value. The circles represent computations with a vessel partial pressure of $u_0 = 8$; the dots represent computations with $u_0 = 16$ and the crosses represent computations with $u_0 = 40$. doi:10.1371/journal.pone.0038597.g017](image)
were strikingly similar with the qualitative and quantitative features much more strongly dependent on the partial pressure of the vessels and their number. In part, this is because after the first few minutes, although one can still see the after-effects of the position of the blood vessels in the spatial distribution of the tracer, as shown in figure 12, the actual magnitude of the variation at any point in time is relatively small. This is because typical diffusion times for tracers, $t = 2D_T$, are an order of magnitude shorter than typical times associated with the binding for tracers, as given by $1/K_{max}$. In real tissue, the vessels are not only highly heterogeneous in their position, but also in their size, vessel partial pressure and vessel blood flow rates. However, the difference in timescales between the diffusion and the chemical kinetics suggests that this heterogeneity is averaged out by the diffusion process and is not detectable on the timescale of the chemical kinetics. The consequence is that fitting a TAC to a partial differential equation model including the full heterogeneity in the distribution and the characteristics of the vessels will result in essentially the same prediction for mean oxygen partial pressure as fitting to a compartment model. While the partial differential equation models that include vascular structures are valuable for allowing the investigation of how changes to the underlying physiological parameters affect the results, this suggests that compartment models will be sufficient in a clinical setting.

### References


### Supporting Information

- **Table S1** Measured values for the physical parameters. (PDF)
- **Table S2** Parameter ranges for non-dimensional quantities. (PDF)

### Author Contributions

Conceived and designed the experiments: AS DL DB AN. Performed the experiments: AS GC DL VM. Analyzed the data: AS GC. Contributed reagents/materials/analysis tools: AS GC DL VM. Wrote the paper: AS.
The Effect of the $G_1$ - $S$ transition Checkpoint on an Age Structured Cell Cycle Model

Gary S. Chaffey*1, David J. B. Lloyd1, Anne C. Skeldon1, Norman F. Kirkby2

1 Department of Mathematics, University of Surrey, Surrey, England, 2 Department of Chemical Engineering, University of Surrey, Surrey, England

Abstract

Knowledge of how a population of cancerous cells progress through the cell cycle is vital if the population is to be treated effectively, as treatment outcome is dependent on the phase distributions of the population. Estimates on the phase distribution may be obtained experimentally however the errors present in these estimates may effect treatment efficacy and planning. If mathematical models are to be used to make accurate, quantitative predictions concerning treatments, whose efficacy is phase dependent, knowledge of the phase distribution is crucial. In this paper it is shown that two different transition rates at the $G_1$ - $S$ checkpoint provide a good fit to a growth curve obtained experimentally. However, the different transition functions predict a different phase distribution for the population, but both lying within the bounds of experimental error. Since treatment outcome is effected by the phase distribution of the population this difference may be critical in treatment planning. Using an age-structured population balance approach the cell cycle is modelled with particular emphasis on the $G_1$-$S$ checkpoint. By considering the probability of cells transitioning at the $G_1$-$S$ checkpoint, different transition functions are obtained. A suitable finite difference scheme for the numerical simulation of the model is derived and shown to be stable. The model is then fitted using the different probability transition functions to experimental data and the effects of the different probability transition functions on the model’s results are discussed.

Citation: Chaffey GS, Lloyd DJB, Skeldon AC, Kirkby NF (2014) The Effect of the $G_1$ - $S$ transition Checkpoint on an Age Structured Cell Cycle Model. PLOS ONE 9(1): e83477. doi:10.1371/journal.pone.0083477

Editor: Raffaele A. Calogero, University of Torino, Italy

Received September 5, 2013; Accepted November 13, 2013; Published January 9, 2014

Copyright: © 2014 Chaffey et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: GC’s contribution was funded by an Engineering and Physical Sciences Research Council (EPSRC) supported Doctoral Training Centre, funded through EP/P505755/1. There was no other external funding for this project. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: g.chaffey@surrey.ac.uk

Introduction

The cell cycle is an ordered set of events that a cell undergoes from its birth until it divides into two daughter cells [1]. In eukaryotic cells the cell cycle may be broken down into four distinct phases, namely $G_1$, $S$, $G_2$ and $M$. After birth, a cell enters the longest of the phases, the $G_1$ (Gap 1) phase, during which the cell takes on nutrients needed to complete the rest of the cycle. Once the cell has absorbed enough nutrients it may proceed round the cell cycle leaving the $G_1$ phase and entering the $S$ (Synthesis) phase. Not all cells leave the $G_1$ phase to enter the $S$ phase, a number of cells enter a quiescent period where they remain viable but leave the cell cycle for a short time, these cells enter the $G_0$ (Gap 0) phase. During the $S$ phase a cell replicates its DNA, at the end of which they have effectively doubled their DNA content. Once DNA synthesis is completed the cell enters the $G_2$ (Gap 2) phase. During the $G_2$ a cell grows in size and prepares for mitosis. Upon leaving $G_2$ the final phase $M$ (Mitosis) is entered. It is during the mitotic phase that the cell divides, producing two daughter cells. Due to the processes involved in cell division, cells in the $M$ phase are especially vulnerable to radiotherapy. It should be noted that the $M$ phase may be broken down further into several sub phases, however this is of no consequence for the model discussed herein. The actual length of the cell cycle is variable, this variability mainly occurs in the length of time cells spend in the $G_1$ phase which is governed by the way in which cells ‘transition’ from the $G_1$ phase to the $S$ phase [2]. Once a cell commits itself to DNA synthesis (i.e. enters the $S$ phase) it must continue the cell cycle until division is complete, the ‘transition’ from the $G_1$ phase to the $S$ phase is irreversible.

Chemotherapy drugs can be divided into several types, each of which target a specific process within the cell cycle such as RNA synthesis or cell division. Hence the efficacy of many chemotherapy drugs, e.g. [3], [4] and [5], is dependent on the cell cycle phase. The radiosensitivity of cells is also phase dependent, e.g. [6], [7] and [8], with cells in the $M$ (mitotic) phase having their chromosomes arranged in a line prior to separation making them particularly sensitive to ionising radiation. Due to the phase dependent nature of chemotherapy drugs and radiotherapy knowledge of how the cells progress through the different phases is crucial.

There have been a number of mathematical models developed for populations of cells progressing round the cell cycle. Systems of ordinary differential equations may be used to model the growth kinetics of populations of cells however these are too simplistic to capture the intrinsic properties of the cell cycle, but are often an invaluable first step in understanding the kinetics of a population of cells. To adequately model crucial properties of a population of cells such as age, mass or DNA distribution a system of partial differential equations is needed.

Many partial differential equation models share the same fundamental population balance structure as detailed in [9], [10] and [11]. These models may broadly be grouped in terms of which
property of the cell is used to structure the model, the main properties used being DNA ([12], [13], [14], [15] and [16]), age ([17], [18], [19] and [20]) and mass ([17], [21], [19]).

There are advantages of using a DNA or mass structured model in as much that these quantities may be easily determined experimentally, however such a model contains no information about the age of a particular cell and as such it is possible for cells to remain in the cycle for an infinite amount of time. By use of an age-structured model it is possible to control the length of time a cell may remain in the cell cycle, in particular the G1 phase. Another advantage of age structuring is that, if growth rates and nutrient uptake rates for a given cell line are known, it is possible to determine the mass and DNA content of a cell from its age, however given the cells DNA content or mass it is not possible to determine a cell’s age as there is not a one-to-one mapping between age and DNA or mass.

Analysis has been undertaken to determine the existence and stability of steady size/DNA distributions [22] which may occur under specific circumstances using an age structured model.

Population balance models have been used not only on healthy, unperturbed cell lines but also to model the effects of various treatments to cancer cell populations [15], [18], [16] and [23].

In this paper, an age structured cell cycle model is considered together with two different functions governing the movement between the G1 and S phases. Whilst, different functions have been used in the past [18], [20] and [24] little has been done to study the effects of different functions on the phase distributions of cells. It is shown that it is possible to obtain very similar growth curves using different transition functions with the fundamental difference being in the phase distributions for the cells. Although the differences in the phase distributions lie within the range of experimental error for many techniques such as conventional flow cytometry it may be significant in terms of treatment optimisation.

The purpose of this paper is to understand how different transition rules may effect the phase distribution of the cells and that whilst the motivation for this analysis is the phase dependent nature of certain treatments these have not been included within the model.

This paper is outlined as follows. The age structured model is presented in Section 1 together with a brief overview of the derivation of a generalised transition function in Section 2. Two specific transition functions are then considered. In Section 3 the numerical scheme used for computations is derived. Section 4 sees the model outline as follows. The age structured model is presented in Section 1 together with a brief overview of the derivation of a generalised transition function in Section 2. Two specific transition functions are then considered. In Section 3 the numerical scheme used for computations is derived. Section 4 sees the derivation of a generalised transition function in Section 2. Two specific transition functions are then considered. In Section 3 the numerical scheme used for computations is derived. Section 4 sees

Model Outline

1 Age structured model

The model considered in this paper is divided into three, age-structured sections, G1b, G1b and MAIN as depicted in Figure 1. The MAIN compartment contains cells in the S, G2 and M phases of the cell cycle, it is at the end of this compartment cell division occurs.

The G1b section contains cells which have just undergone division. Cells that are in G1b are not able to progress further round the cell cycle until a fixed time period has elapsed, this represents the minimum age a cell can start replicating its DNA. This is biologically realistic as new cells are normally unable to immediately start replicating their DNA. Once cells have progressed to G1b they undergo transition to the MAIN compartment at a rate $b(v)$, which is often a function of how long the cell has spent in G1b. It may also be a function of other factors which effect a cell’s progression round the cell cycle such as nutrient levels, the presence of certain drugs, temperature etc. The MAIN compartment is of fixed duration and can be thought of as merely a time delay from when a cell leaves G1b until cell division and entry of the new daughter cells into G1b. All compartments within this model are of a limited duration, the MAIN and G1b compartments are of a fixed duration and the duration of G1b varies from zero to some maximum value, $T_{G1b}$. Biologically, any cells remaining in G1b at the end of $T_{G1b}$ would either die or enter a quiescent phase. Cells in a quiescent phase may be able to rejoin the cycle at a later time. Neither of these scenarios is modelled here.

In this model the non dimensionalised equations governing the population density of cells $n$ in each phase are given by

$$\frac{\partial n_{G1b}}{\partial t} + \frac{\partial n_{G1b}}{\partial \tau} = 0,$$

$$\frac{\partial n_{G1b}}{\partial t} + \frac{\partial n_{G1b}}{\partial \tau} + b(v)n_{G1b} = 0,$$

$$\frac{\partial n_{MAIN}}{\partial t} + \frac{\partial n_{MAIN}}{\partial \tau} = 0.$$
condition is not important, however for completeness it may be assumed there is a uniform feed of cells into the start of the cell cycle for the first $k$ hours,

$$n_{G_{1a}}(t,0)=c, \quad t<k \text{ hours.} \quad (5)$$

This model is of a similar structure to most population balance age-structured models such as those presented in [18] and [20] amongst others. In [20] the MAIN phase is split into three parts $S, G_2$ and $M_1$, but since our focus is on the total cell population and the fraction of cells in $G_1$, this difference has no impact. A further difference is in the way that [20] model the transition from $G_1$, and this will be discussed in greater detail below. In [18], in addition, the $G_1$ phase is modelled as a single compartment rather than divided into two, $G_{1a}$ and $G_{1b}$.

2 G$_1$-S Transition functions

The probability of a cell leaving the $G_{1b}$ phase and entering the $S$ phase via the transition rule is given by some probability distribution function $f(x)$ where $x$ is the variable that determines how likely cells are to undergo transition. Figure 2 gives a graphical representation of such a probability distribution function with phase age $\tau_{G_{1a}}$ acting as the variable controlling the transition probability. Note that phase age is the length of time a cell spends in a particular phase, For the rest of this paper the subscripts have been removed from the age variable for ease and only used in the case of any ambiguity as to the phase referenced.

If $\tau$ varies by a small amount, $\delta\tau$, then the probability of cells whose age is between $\tau$ and $\tau+\delta\tau$ transitioning can be approximated by $f(\tau)\delta\tau$. Assuming all cells are capable of transitioning given enough nutrients, the total area under the probability distribution curve is one. Therefore the probability that a cell of age $\tau$ has not yet transitioned is given by $1 - \int_0^{\tau} f(\tau)\,d\tau$. So the fraction of cells, who have not gone through transition, who go through transition when their age changes from $\tau$ to $\tau+\delta\tau$ is given by

$$f(\tau)\delta\tau \over 1 - \int_0^{\tau} f(\tau)\,d\tau. \quad (6)$$

Another way of considering the number of cells going through transition is via a transition rate $h(\tau)$. If the fraction of cells who leave in the time period $[\tau,\tau+\delta\tau]$ is given by $h(\tau)\delta\tau$, then by definition this must be equal to equation (6). Therefore, in the limit $\delta\tau \to 0$,

$$h(\tau) = { f(\tau) \over 1 - \int_0^{\tau} f(\tau)\,d\tau}. \quad (7)$$

since a cell ages at the same rate as time passes $t(\tau)=\tau-c$ where $c$ is a constant therefore $d\tau = dt$ hence equation (7) simplifies to

$$h(\tau) = { f(\tau) \over 1 - \int_0^{\tau} f(\tau)\,d\tau}. \quad (8)$$

If the cumulative probability of cells transitioning, $F(\tau)$, is considered then equation (8) may be expressed as

$$h(\tau) = { F'(\tau) \over 1 - F(\tau)}. \quad (9)$$

where the dash notation denotes the derivative with respect to $\tau$. It is this form of the transition rate which will be used herein.

2.1 Specific transition rules. In this paper, we consider two different transition functions, the first assumes that the transition rate is constant, $h=\frac{c}{S}$, and is therefore independent of the time spent in the $G_{1b}$ phase. Note the transition rate $h=\frac{c}{S}$ corresponds to a cumulative probability of transition given by $1-e^{-\frac{c}{S}}$. This is the same form of transition discussed in [18]. This transition rule is not biologically realistic as it implies all cells in $G_{1b}$ have an equal probability of progressing to the $S$ phase regardless of how long they have spent acquiring nutrients and preparing for DNA synthesis.

The second form of transition function that we consider is a sigmoidal transition function. This seems biologically reasonable since this implies that the probability of cells progressing to the $S$ phase immediately after entering $G_{1b}$ is low due to the limited amount of nutrients they have absorbed. Once the mass of nutrients absorbed reaches some critical value then the probability of transition is likely to increase considerably, however there will always be a few cells which do not progress to the $S$ phase regardless of nutrient uptake, thus the sigmoidal function attains a maximum value just under one. It should be noted that a sigmoidal cumulative probability function is in keeping with the sigmoidal transition function. This seems biologically reasonable to expect that the cumulative distribution function should be non-zero at $\tau_{G_{1a}}=0$. Furthermore, as discussed earlier, some cells will not transition and enter a quiescent state so the cumulative distribution for $G_{1b}$ remains less than one for all $\tau_{G_{1a}}$. Therefore, the cumulative distribution function given by

$$F(t;\tau)=1 - \frac{1}{1 + e^{\frac{C_{G_{1a}}(t)}{C_{max}} \frac{\tau}{\tau_{max}}}} \quad (10)$$

is considered. Here, $\theta$ is related to the maximum and minimum values of the cumulative distribution function and $C_{max}$ is related to the steepness of the sigmoidal function and $C_{G_{1a}}(t;\tau)$ represents
the amount of glutamine a cell of age $\tau_{ib}$ has absorbed at time $t$. It then follows that, for $\theta$ sufficiently large,

$$h(t,\tau) = \frac{\theta}{C_{\text{max}}} \frac{\partial C_{i}(t,\tau)}{\partial \tau} e^{-\frac{\theta t}{C_{\text{max}}}} + \frac{1}{1 + e^{-\frac{\theta t}{C_{\text{max}}}}}. \quad (11)$$

It is reasonable to assume that the rate of change of glutamine is constant, provided there is a high amount of glutamine available. By making this assumption then $\frac{\partial C_{i}(t,\tau)}{\partial \tau} = R$ and $C_{i} = \tau R$ (It is assumed that the cell has not taken any glutamine prior to entering the $G_{1b}$ phase, i.e. $C_{i} = 0$ at $\tau = 0$). Hence,

$$h(t) = \frac{\theta R}{C_{\text{max}}} e^{-\frac{\theta t}{C_{\text{max}}}} + \frac{1}{1 + e^{-\frac{\theta t}{C_{\text{max}}}}}. \quad (12)$$

The corresponding non-dimensional form of this equation is given by

$$h(t) = \frac{t - \frac{1}{\theta}}{1 + e^{t - \frac{1}{\theta}}} \quad (13)$$

which only has the single parameter $\theta$ which needs to be fitted. In [28] the following expression for the fraction of cells of age $\tau$ remaining in the $G_{1b}$ phase, $n(t,\tau)$, for a given intra cellular glutamine concentration $C_{Gib}(t,\tau_{ib})$ is proposed

$$n_{Gib}(t,\tau_{Gib}) = m_{Gib}(t - \tau_{Gib}, 0) = \left(\frac{C_{Gib}(t,\tau_{Gib}) - S_{\text{Max}}}{S_{\text{Max}}}\right)^{2}. \quad (14)$$

where $S_{\text{Max}}$ is the maximum glutamine content a cell can have before being forced to go through transition. This leads to the transition function

$$h(t,\tau_{Gib}) = \frac{2}{S_{\text{Max}} - C_{Gib}(t,\tau_{Gib})} \frac{\partial C_{Gib}(t,\tau_{Gib})}{\partial \tau_{Gib}}. \quad (15)$$

Note $\frac{\partial C_{Gib}(t,\tau_{Gib})}{\partial \tau_{Gib}}$ is assumed to always be $\geq 0$ so that the cumulative glutamine never decreases. It can be seen that when $C_{Gib}(t,\tau_{Gib}) = S_{\text{Max}}$, the probability of transition becomes infinite. Despite this singularity at $C_{Gib}(t,\tau_{Gib}) = S_{\text{Max}}$, this transition function still provides a very good fit to experimental data [20]. The reasons why this is the case are discussed below.

**Numerical Methods**

The system of differential equations governing the simplified system described in Section 1 may be solved analytically for specific initial conditions and short time intervals. However, in order to be able to study and manipulate the model for different transition functions for longer time intervals involving many cell cycles it is necessary to use numerical techniques.

3 Derivation of Numerical scheme

In this section a finite difference scheme analogous to the Lax-Wendroff scheme is derived. The Lax-Wendroff scheme was chosen as it is a second order explicit method and as such yields high accuracy for relatively large time steps where there is a rapid change or discontinuity such as the initial flow of cells into the main cycle.

For the $G_{1b}$ phase equation (2) may be written as

$$n_{i} + n_{e} = -hn, \quad (16)$$

Note for ease the time and age dependence has been omitted together with the phase subscript. Subscripts now denote the partial derivatives. Also $h$ is a function of $\tau$ only, furthermore, if the sigmoidal form of the transition rule given in equation (13) is used then

$$h_{i} = h - h^{2}. \quad (17)$$

Rearranging and differentiating equation (16) gives

$$n_{i} = -n_{e} - hn_{e}, \quad (18a)$$

$$n_{e} = -n_{i} - hn_{i}, \quad (18b)$$

$$n_{e} = -n_{i} - hn_{i} - h_{n}. \quad (18c)$$

Which, upon using the Taylor expansion together with (17) yields

$$n(t + \delta t, \tau) = n(t) - \delta th + \frac{(\delta t)^{2}}{2} h + n(t - \delta t + (\delta t)^{2} h) + O(\delta t^{3}). \quad (19)$$

Finally, standard formulae for the first and second derivatives of $n$ with respect to $\tau$ are used, namely

$$\frac{\delta n}{\delta \tau} |_{i,j} = \frac{n_{i+1,j} - n_{i-1,j}}{2\delta \tau}. \quad (20)$$

$$\frac{\delta^{2} n}{\delta \tau^{2}} |_{i,j} = \frac{n_{i+1,j} - 2n_{i,j} + n_{i-1,j}}{(\delta \tau)^{2}}. \quad (21)$$

where $n_{i,j}$ is the cell density of cells aged $[\tau_{j},(j+1)\tau_{j})$ in the time interval $[t_{j},(j+1)t_{j})$ where $t_{j}$ and $\tau_{j}$ are the length of the discretised elements. This leads to the finite difference scheme

$$n_{i+1,j} = n_{i,j} \frac{1 - (\delta \tau)^{2}}{2(\delta \tau)^{2}} - \delta th_{i,j} + \frac{(\delta \tau)^{2}}{2} h_{i,j}$$

$$+ n_{i,j+1} \frac{(\delta \tau)^{2}}{2(\delta \tau)^{2}} - \frac{\delta t}{2\delta \tau} + \frac{(\delta \tau)^{2}}{2(\delta \tau)^{2}} h_{i,j}$$

$$+ n_{i,j-1} \frac{(\delta \tau)^{2}}{2(\delta \tau)^{2}} + \frac{\delta t}{2\delta \tau} + \frac{(\delta \tau)^{2}}{2(\delta \tau)^{2}} h_{i,j}. \quad (22)$$

Because of the `dispersive' nature of any numerical difference scheme if $\delta \tau \neq \delta t$ additional errors are introduced at each time step. For example if at $t = 0$ all cells are age zero and the age step is set to $\varepsilon$ and the time step set to $\frac{1}{\theta}$, then after evolving the system for one time step there would be cells whose age is $\varepsilon$, this clearly makes
no sense. Similarly if the time step is set to 2e after one step there are no cells present whose age is 2e since t ≤ e for all cells. Hence, additional interpolation is required if the age and time steps are not equal. By setting δt = δt = x equation (24) becomes

$$n_{i+1,j} = n_{i,j} \left\{ \left( \frac{t^2}{2} - 3 \right) h_{i,j} \right\} + n_{i,j+1} \left( \frac{x^2}{2} h_{i,j} \right) + n_{i,j-1} \left( 1 - \frac{x}{2} h_{i,j} \right). \quad (23)$$

3.1 Stability of the numerical system. For a numerical scheme to produce accurate solutions to a partial differential equation, not only must the error at each time step be small enough, any errors must not grow exponentially, i.e. the numerical scheme must also be stable. If the nutrient supply is unlimited and uptake is uniform then the cell cycle may be simplified into two ‘phases’, $G_0$ on it’s own and the remaining phases all put together. A two compartment model is not suitable for analysing the dynamics of a population of cells as too much information is lost by combining the MAIN phase and $G_0$ phases of the model discussed in Section 1, in particular the timing of the cell division. However, a two compartment model is sufficient for conducting a stability analysis. Once the system has reached steady growth (i.e. maximum age in $G_0$), then at time $t_0$ then let cells in phase $X$ of age $e_i(t_0)$ be denoted by $X_i$. Also let all cells in phase $X$ in the time interval $\varepsilon(t_0, t_1)$ be denoted by $X_m$, where $X_m$ is now a column vector. Also assume the time line is moved such that at $t = t_0$, $t = 0$, where $t$ is the time used for the purposes of the subscript; for convenience the ‘$i’ notation is now dropped.

Construction of time step matrix. Let the maximum durations of the $X$ and $Y$ phases be $N_{t_0}$ and $K_{t_0}$ respectively then at time $t = t_0$,

$$X_0^0 \text{ cells entering } X,$$

$$X_0^{N-1} \text{ cells in } X \text{ dying due to old age at the next time step,}$$

$$Y_0^0 \text{ cells entering } Y,$$

$$Y_0^{K-1} \text{ cells leaving } Y \text{ and doubling at the next time step.}$$

Clearly,

$$Y_0^0 = 2Y_0^{K-1}. \quad (25)$$

Also the cells entering $Y$ are a function of the cells who were in $X$ at the previous time step, therefore

$$Y_0^0 = h(X_{t_0-1}), \quad (26)$$

where $h(t)$ is the probability of transition from $X$ to $Y$, since nothing happens to the cells during their time in $Y$, it can be thought of as merely a time delay phase, therefore

$$Y_0^j = Y_0^{j-1} + Y_0^{j-2} \quad \text{for } 1 \leq j \leq K. \quad (27)$$

Note, the inequality is strictly less than $K$ as cells of age $K_{t_0}$ have undergone division and the offspring are now in $X_0^0$.

Assuming a finite central difference scheme is used for calculating the cell densities in the $X$ phase then

$$X_0^j = f(X_{t_0-1}^j, X_{t_0-1}^{j-1}, X_{t_0-1}^{j-1}, Y_{t_0-1}^{j-1}, 4) \quad \text{for } 1 \leq j \leq N, \quad (28)$$

and

$$X_0^N = f(X_{t_0-1}^N, X_{t_0-1}^{N-1}). \quad (29)$$

From equations (25) and (27) it is clear that

$$X_0^j = 2Y_0^{j-1} \quad \text{for } 1 \leq j \leq K. \quad (30)$$

Now using equation (26) yields

$$X_0^j = 2h(X_0^j), \quad (31)$$

Equations (25–29) may be expressed in matrix notation as

$$\begin{pmatrix}
X_0^0 \\

X_1^0 \\

X_2^0 \\

\vdots \\

X_{N-1}^0 \\

Y_0^0 \\

Y_1^{K-1}
\end{pmatrix} = M
\begin{pmatrix}
X_0^0 \\

X_1^0 \\

X_2^0 \\

\vdots \\

X_{N-1}^0 \\

Y_0^0 \\

Y_1^{K-1}
\end{pmatrix}, \quad (32)$$

where $M$ is an $(N+K) \times (N+K)$ matrix. To prove the numerical scheme is stable it is sufficient to show [29] that the norm of $M$ in equation (32) satisfies

$$\| M \| \leq 1 + \kappa x, \quad (33)$$

where $\delta t = \delta t = x$ and $\kappa$ is a constant independent of $x$. It can be shown that if the trapezium rule is used for approximating equation (26) then the norm of $M$ is given by

**Table 1.** Parameters from [20].

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Notation</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum age in $G_0$ phase</td>
<td>$T_{G_0}$</td>
<td>2.5 hours</td>
</tr>
<tr>
<td>Maximum age in $G_0$ phase</td>
<td>$T_{G_0}$</td>
<td>10 hours</td>
</tr>
<tr>
<td>Maximum age in $S$ phase</td>
<td>$T_S$</td>
<td>5 hours</td>
</tr>
<tr>
<td>Maximum age in $G_0 + M$ phase</td>
<td>$T_{G_0 + M}$</td>
<td>4 hours</td>
</tr>
</tbody>
</table>

![Figure 3](https://www.plosone.org/content/fig/figure3/figure3.jpg)
For the transition functions considered $h$ is monotonically increasing so

$$\sum_{j=1}^{N-2} h(j) + \frac{N}{2} (h(0) + h(N-2)) \leq \int_{0}^{X_{\text{max}}} h(\tau) d\tau, \quad (35)$$

it is therefore sufficient to show $\int_{0}^{X_{\text{max}}} h(\tau) d\tau$ remains bounded. For the sigmoidal transition rule

$$\int_{0}^{X_{\text{max}}} \frac{e^\left(-\frac{\tau}{2}\right)}{1+e^\left(-\frac{\tau}{2}\right)} d\tau = \ln \left(1+e^{-X_{\text{max}}/2}\right). \quad (36)$$

which for typical $\theta$ values this is approximately equal to

$$\ln \left(1+e^{-X_{\text{max}}/2}\right) \approx 1+e^{-X_{\text{max}}/2} \leq 2. \quad (37)$$
Thus, in all cases $\|M\|$ remains bounded. In most cases $(X_{\text{max}} - \frac{\theta}{2})^2 < 1$, this leads to a stronger constraint on the bound i.e., $\|M\| \leq 2$.

Results

In Section 4 it is shown that regardless of whether a constant or a sigmoidal transition rule is used, it is possible to fit the model to a growth curve from experimental data. It is then shown in Section 5 that whilst the different transition functions result in the same growth curve, the fraction of cells in each phase differs.

4 Model validation

Experimental data from [25] was chosen and concerns a batch experiment which was conducted using a mouse-mouse hybrid-oma cell line (mm321). In this experiment 28% of the starting cell population did not divide but remained viable, 36% of the starting population were evenly distributed in the $S$ phase of the cell cycle and the remaining 36% were initially at the beginning of the $G_{1b}$ phase. For the purposes of modelling it was assumed the cells starting in the $G_{1b}$ phase were of a phase age between zero and two hours. The numerical scheme described in Section 3, was implemented using both sigmoidal and constant transition rules. Parameters for the length of different phases were taken from [20], and are stated in Table 1. The $\theta$ and $c$ parameters were allowed to vary in the sigmoidal and constant transition rules respectively, until a best fit had been obtained. Several starting values for $\theta$ and $c$ were used in the optimizations of the fits to ensure the global best fits had been found for each transition rule and that the results were not a local minimum. Optimizations were carried out using Matlab’s [30] least squares curve fitting algorithm \textit{lsqcurvefit}. The Matlab code for these optimizations is available from [31].

As can be seen in Figure 4, both the constant transition rule (Figure 4a) and the sigmoidal rule (Figure 4b) provide a good fit to the experimental data resulting in residual norm values of 0.1 and 0.2 respectively. The parameters in Table 1 were varied by

![Figure 6. Constant transition function (a) with the corresponding cumulative probability of transition (b) as a function of $G_{1b}$ age.](doi:10.1371/journal.pone.0083477.g006)

![Figure 7. Sigmoidal transition function (a) with the corresponding cumulative probability of transition (b) as a function of $G_{1b}$ age.](doi:10.1371/journal.pone.0083477.g007)
Different values for the Table 1 parameters resulted in different values for the fitted parameters ($h$ and $c$) but did not significantly change the goodness of the fit shown in Figure 4 with no residual norms exceeding 0.2. Note that the model did not impose any restrictions on the available nutrients, indicating that was not a limiting factor for cell growth over the course of the experiment. This suggests, that if population growth is the only concern, that a constant transition rule is sufficient.

5 The effect of the transition function

Although the effect of the different transition rules is not apparent in the fitting to the experimental growth curve, here we show that the transition rule does impact on the phase distribution of cells.

In the experimental data used to fit the model the initial population of cells was partially synchronised using a thymidine double block. This partial synchronisation meant the initial population of cells was situated in the $G_1$ phase and the latter part of the $G_2$ phase, $G_{SB}$. It therefore seems reasonable to assume most cells will initially progress round the cycle in a group this would result in the phase distribution being oscillatory. The oscillations would be expected to decay slowly as the synchronicity of the cell population was lost. Such oscillations may be one cause for apparent ‘errors’ in phase distributions obtained from such experiments as the timing of observations would need to occur at known positions on the oscillation, the period of which may not be known. To fully appreciate the differences these transition functions have on the underlying model properties the percentages of cells in each compartment may be compared once transient oscillations have decayed and the system has reached a steady state of phase distributions. The time scale required for the transient oscillations to have decayed sufficiently is of the order of 500 hours and as such it is not feasible to obtain experimental data.

In order to investigate this, the mathematical model was numerically integrated using the same parameters and initial conditions used in Section 4 for long enough that a steady phase distribution had been obtained. The results are shown in Figure 5. These two sets of results differ in two key ways. Firstly, both simulations initially show an oscillation in the phase distribution, however the rate of decay of the oscillations depends on the transition function chosen, with the oscillations decaying much more slowly for a sigmoidal transition function. The difference in the decay rates may be appreciated by considering the area under the cumulative probability distribution function controlling the transition function places 47.0% of cells in the $G_1$ phase, whilst the constant transition function places 47.0% of cells in the $G_1$ phase.

As mentioned previously the efficacy of chemotherapy treatments and the radioresistance of cells varies according to a cell’s position in the cell cycle. Since the relationship between cell phase and efficacy may be non-linear a small difference in phase distribution may produce a large change in the efficacy of treatments resulting in the model producing results outside the bounds of experimental error. Therefore, the difference in the phase distributions produced by this model, using the different transition functions, will effect the model’s ability to accurately represent the effects of a given treatment on a population of cells. Consequently, it is important to ascertain the correct transition function if such models are to be used to give a quantitative prediction of the cell population’s response to treatments.

Improvements in techniques may reduce the level of potential error in phase distributions obtained experimentally, this may allow some transition functions to be discounted.

It may also be possible to rigorously derive the form of the transition function for a population of cells by considering the chemical kinetics of a single cell [26].

Whilst there is no consensus on the error on cell phase distributions obtained using flow cytometry [32] the difference in phase distributions produced by the model with the different transition rules lie within the typical bounds of current experimental error ([33], [34] and [32]). As noted in Section 5 the difficulty of measuring the phase distribution may be compounded by underlying oscillations induced by the blocking. Thus, the form of the probability distribution function controlling the $G_1 - S$ checkpoint in an age structured population balance model has little impact on the models ability to fit to experimental data. The lack of effect of the form of the probability transition function explains why the quadratic transition function used in [20] fitted experimental data despite having a singularity. As such a simplified transition function may be used to gain a qualitative understanding of the dynamics of a population of cells.

Author Contributions

Conceived and designed the experiments: GSC DJBL ACS NFK. Analyzed the data: GSC DJBL ACS. Contributed reagents/materials/analysis tools: GSC DJBL ACS. Wrote the paper: GSC DJBL ACS.

References