MODEL BASED OPTIMAL CONTROL OF GENE DELIVERY SYSTEMS

A Doctoral Thesis

by

Elnaz Jamili

In partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

at

University College London

Department of Chemical Engineering

University College London

2018
Dedicated

to

my lovely parents and my gorgeous brother
Declaration

‘I, Elnaz Jamili, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.’

Jamili, E.
Abstract

After more than two decades of intensive investigations, gene therapy has made impressive recent progress and is now among the most promising strategies for treating genetic disorders. However, a major challenge currently facing a clinical translation of gene-based therapy is the lack of an optimal gene delivery vector. This PhD thesis aims to investigate the application of mathematical modelling techniques, coupled with the optimal control theory, in gene delivery systems in order to improve the pharmacological effects while minimising the toxicological responses.

The first contribution of this work presents an innovative approach based on the optimal control strategy for optimising the process of gene delivery. The methodology developed in this work highlights the advantages of process modelling and model analysis, contributing towards a detailed quantitative understanding of the system, while aiming for the optimal control of such systems. An integrated pharmacokinetic/pharmacodynamic (PK/PD) model-based optimal control algorithm was developed for non-viral siRNA delivery. This aims at incorporating the dynamics of the delivery process while simultaneously considering the main multi-objective optimisation issues, such as efficacy and toxicity. The framework presented in this thesis provides an efficient model-based platform for making decisions under uncertainty, which is lacking for gene delivery systems. As part of the presented approach, the model uncertainty that comes from variability in cell division time was analysed and the developed control strategy was tested in the presence of uncertainty.

The proposed methodology was also tested by using in vivo clinical data for gene therapy in patients with haemophilia B. Haemophilia B is a genetic bleeding disorder resulting from a deficiency or dysfunction of a protein called factor IX, which is critical for blood-clotting. In this work, a modelling framework is proposed to predict the physiological response of a subject affected by type B haemophilia to a dose of vector. The results from this study demonstrate a good prediction of the model. The PK/PD parameters were individually estimated for each patient in a dose-independent manner for a personalised gene therapy, while a population modelling approach was investigated to guide initial dose selection.
The modelling framework being developed in this thesis should be extended in the future to include the spatial distribution of the genetic materials resulting in a system of reaction-diffusion partial differential equations (PDEs). To this end, the last part of the thesis presents a novel theoretical framework for parameter estimation of partial differential equations in a complex geometrical domain using an artificial neural network (ANN) algorithm. This work will provide a stepping stone on the pathway for developing a reaction-diffusion model paving the way for further investigation of the effect of cellular geometry in the diffusion of genetic materials by taking into account the cellular architectures.
Impact Statement

It is almost 30 years since the first authorised gene transfer trial took place at the National Institutes of Health (NIH) in 1989. Gene therapy has fascinated clinicians, scientists and the general public because it holds the promise to transform medicine by treating a wide range of diseases at their genetic roots. However, gene therapy still brings huge concerns about safety and efficacy. It is on this topic that this thesis is based.

The findings of this research demonstrate that the theory of control can be applied to gene delivery systems with relative success. The model-based optimisation approaches provide an effective decision-making platform for gene delivery systems, allowing for optimal infusion profile to be computed while balancing the trade-offs between efficacy and toxicity.

This thesis serves as a starting point in developing automation technologies in the field of gene therapy, paving the path for further advancements in the next-generation delivery device systems capable of capturing the complexity of in vivo conditions. It is envisaged that this work triggers enthusiasm for development of a gene delivery system that integrates an infusion pump with a control system, which can further assist in the optimisation of the process.

The results presented in this thesis also lend further support to the initial dose selection for gene delivery. Together, these findings could provide clinicians with better tools to design more effective treatment plans, which can be tailored to maximise efficacy while minimising toxicity for individual patients.
Acknowledgements

First and foremost, I would like to thank God for being a source of hope, strength and inspiration.

I would like to express my sincere gratitude to my PhD supervisor, Dr Vivek Dua, for his continuous support, valuable guidance, patience, and consistent encouragement, without which this work would not have been possible.

I would also like to thank Professor Amit Nathwani for sharing the clinical data of their patients, which was used in this work for modelling purposes.

A special word of thanks goes to all my friends and colleagues, Vassilis, Ernie, Asif, Matt, Shade and Mayowa, for their help and support.

Finally, and from my heart, I would like to express my very deep respect and special appreciation to my beloved parents, Mr Ebrahim Jamili and Mrs Shahla Asimi, for their continued love, support and encouragement. I would also like to express my deepest gratitude to my gorgeous brother, Mr Reza Jamili, who has been always supportive. Words cannot express how grateful I am to my family. Without their unconditional love and support, I would not be able to complete my PhD.
Dissemination

JOURNAL PUBLICATIONS


Jamili, E. and Dua, V. Mathematical Modelling of Gene Delivery in Patients with Haemophilia B. *in preparation*.


CONFERENCE PAPER


TALKS


Jamili, E. “Modelling and Optimal Control for Delivering of siRNAs”. PSE@ResearchDayUK. Department of Chemical Engineering. Imperial College London, UK, July 12, 2016.


POSTER PRESENTATIONS

Jamili, E. and Dua, V. “Incorporating Time Constraints in Model Based Control of siRNA Delivery”. American Institute of Chemical Engineers (AIChE) Annual Meeting, San Francisco, CA, USA. Nov 14, 2016.


Jamili, E. and Dua, V. “Incorporating Time Constraints in Model Based Control of siRNA Delivery”. ChemEngDayUK 2016, Department of Chemical Engineering, University of Bath, UK. Mar 31-Apr 1, 2016.

Jamili, E. and Dua, V. “Modelling and Optimal Control of Non-Viral siRNA Delivery”. American Institute of Chemical Engineers (AIChE) Annual Meeting, Salt Lake City, UT, USA. Nov 11, 2015.

Jamili, E., Stamatakis, M. and Dua, V. “Modelling and Optimal Control of Non-Viral siRNA Delivery”. ChemEngDayUK 2015, Department of Chemical Engineering, University of Sheffield, UK. Apr 8, 2015.

Contents

Declaration .................................................................................................................. 5
Abstract ....................................................................................................................... 7
Impact Statement ........................................................................................................ 10
Acknowledgements ..................................................................................................... 12
Dissemination .............................................................................................................. 14
Contents ....................................................................................................................... 17
List of Figures ............................................................................................................. 22
List of Tables .............................................................................................................. 26
Abbreviations .............................................................................................................. 27

1 INTRODUCTION ........................................................................................................ 31
   1.1 Background and Motivation .................................................................................. 31
   1.2 Research Objectives ............................................................................................. 34
      1.2.1 Optimal Model-Based Control of Non-Viral siRNA Delivery ................. 35
      1.2.2 Mathematical Modelling of Gene Delivery in Patients with Haemophilia B ............................................................. 35
      1.2.3 Parameter Estimation of Partial Differential Equations using Artificial Neural Network ................................................................. 36
   1.3 Thesis Structure .................................................................................................. 36

2 REVIEW OF GENE DELIVERY SYSTEMS .............................................................. 39
   2.1 Introduction .......................................................................................................... 39
   2.2 Gene Therapy and its Current Trends ................................................................. 40
   2.3 Gene Delivery Systems ........................................................................................ 42
   2.4 Biological Responses ........................................................................................... 45
   2.5 Modelling of Gene Delivery Systems ................................................................ 49
      2.5.1 Pharmacokinetic Modelling ......................................................................... 50
      2.5.2 Pharmacodynamic Modelling ....................................................................... 52
      2.5.3 Mathematical Modelling of Gene Delivery Systems ................................... 54
2.6 Summary ...................................................................................................................... 56

3 REVIEW OF DYNAMIC SIMULATION AND OPTIMISATION .......................... 58
3.1 Introduction .............................................................................................................. 58
3.2 Overview of Differential Equations ...................................................................... 59
3.3 Optimal Control ...................................................................................................... 60
3.4 Numerical Methods for Solving Differential Equations ..................................... 61
  3.4.1 Fourth Order Runge–Kutta (RK4) ................................................................. 62
  3.4.2 Orthogonal Collocation on Finite Elements (OCFE) ................................. 62
  3.4.3 Artificial Neural Network (ANN) ................................................................. 64
3.5 Parameter Estimation of Process Systems ......................................................... 66
  3.5.1 Parameter Estimation of Ordinary Differential Equation Systems ..... 68
  3.5.2 Parameter Estimation of Partial Differential Equation Systems .......... 69
3.6 Summary ...................................................................................................................... 72

4 OPTIMAL MODEL-BASED CONTROL OF NON-VIRAL siRNA DELIVERY ................................................................. 75
4.1 Introduction .............................................................................................................. 75
4.2 Methods ..................................................................................................................... 76
  4.2.1 Mathematical Modelling of siRNA Delivery .............................................. 76
  4.2.2 Experimental Data ....................................................................................... 77
  4.2.3 Pharmacokinetic Modelling ........................................................................ 77
  4.2.4 Pharmacodynamic Modelling and Optimal Control of siRNA Delivery...
    ............................................................................................................................. 78
    4.2.4.1 Gene Silencing Activity ....................................................................... 78
    4.2.4.2 Cytotoxicity ......................................................................................... 79
    4.2.4.3 Optimal Control .................................................................................. 80
4.3 Results ....................................................................................................................... 81
  4.3.1 Bolus Injection ............................................................................................... 85
  4.3.2 Optimal Control of siRNA Delivery ............................................................ 87
  4.3.3 Incorporating Time Constraints ................................................................... 91
4.4 Conclusions .............................................................................................................. 96

5 MATHEMATICAL MODELLING OF GENE DELIVERY IN PATIENTS WITH HAEMOPHILIA B ................................................................. 99
5.1 Introduction .............................................................................................................. 99
5.2 Methods ..................................................................................................................... 100
5.2.1 Clinical data ................................................................. 100
5.2.2 Pharmacokinetic Modelling ............................................ 101
5.2.3 Pharmacodynamic Modelling ......................................... 102
5.2.4 Incorporating the Toxicological Model ............................ 104
5.3 Results ............................................................................. 105
5.3.1 Parameter Estimation .................................................. 106
5.3.2 Initial Dose Selection ................................................... 120
5.4 Conclusions ................................................................... 123

6 PARAMETER ESTIMATION OF PARTIAL DIFFERENTIAL
    EQUATIONS USING ARTIFICIAL NEURAL NETWORK ............ 125
6.1 Introduction ..................................................................... 125
6.2 Parameter Estimation Methodology ..................................... 128
  6.2.1 Description of the Method ............................................ 128
  6.2.2 ANN Approximation of the Solution .............................. 131
6.3 Numerical Validation of the Model Accuracy ....................... 134
  6.3.1 Problem 1 .................................................................. 134
    6.3.1.1 Parameter Estimation using Uniform Grid .............. 135
    6.3.1.2 Parameter Estimation using Non-Uniform Grid ....... 136
  6.3.2 Problem 2 .................................................................. 137
  6.3.3 Problem 3 .................................................................. 139
  6.3.4 Problem 4 .................................................................. 140
  6.3.5 Problem 5 .................................................................. 141
6.4 Conclusion ....................................................................... 143

7 CONCLUSIONS AND FUTURE WORK .............................. 147
7.1 Summary of Thesis and Key Contributions ......................... 147
  7.1.1 Optimal Model-Based Control of Non-Viral siRNA Delivery .... 148
  7.1.2 Mathematical Modelling of Gene Delivery in Haemophilia Patients 148
  7.1.3 Parameter Estimation of Partial Differential Equations using Artificial
       Neural Network ............................................................. 149
7.2 Future Work ..................................................................... 149
  7.2.1 Control-Relevant Modelling in Gene Delivery .................. 149
  7.2.2 Computational Modelling of Reaction-Diffusion Processes in Gene
       Delivery ........................................................................ 150
7.2.3 Model-Based Optimal Control of Gene Delivery in Patients with Haemophilia B ................................................................. 151

References ......................................................................................................................... 153
List of Figures

**Figure 1.1**: Flowchart illustrating the multi-objective model-based controller algorithm for gene delivery systems. ................................................................. 34

**Figure 2.1**: Intracellular barriers of siRNA delivery using non-viral delivery systems. ..................................................................................... 48

**Figure 2.2**: The one-compartment model with first-order elimination.............. 51

**Figure 2.3**: Schematic representation illustrating the relationship between kinetics and dynamics of a drug. Adapted from Gabrielsson and Weiner (2010). ....... 53

**Figure 3.1**: Collocation on finite elements. $h_{OCFE}$ is the length of the element $fe$ and the length of the domain is defined by the period $t_{fe-1}$ to the final point $t_{Nfe}$. ... 63

**Figure 3.2**: Artificial Neural Network (ANN).................................................................................. 65

**Figure 3.3**: Structure of an ANN node/neuron................................................................. 66

**Figure 3.4**: The estimation problem. .............................................................................. 68

**Figure 4.1**: Two-compartment model. A representation of the compartmentalization where $\theta_i$ represents the process rate constants: $\theta_1$ controls movement out of the endosome and $\theta_2$ controls movement from the cytoplasm to the RNAi machinery................................................................. 78

**Figure 4.2**: Two-compartment model with infusion......................................................... 79

**Figure 4.3**: Block diagram for a model-based optimal control of siRNA delivery. .... 81

**Figure 4.4**: Comparison of the pharmacokinetic experimental results (represented the mean ± S.D.) and the PK model predictions........................................ 82

**Figure 4.5**: Comparison of the cell viability experimental results (represented the mean ± S.D.) and the model predictions........................................ 83

**Figure 4.6**: Schematic illustration of the composite $G_{max}/T_{max}$ model. The $G_{max}$ and $T_{max}$ values were estimated to be 21.2 % and 71.7 %, respectively; $GC_{50}$ and $TC_{50}$ are 15.4 nM and 94.9 nM, respectively, and the exponent is 7. ............. 83

**Figure 4.7**: Sensitivity analysis of the model parameters. .............................................. 84

**Figure 4.8**: Pharmacokinetic and pharmacodynamic responses to bolus injections of siRNA therapeutics after start of treatment with total injected doses of 80, 100, 150, and 250 nM. (a) Time profile of siRNA concentration in the endosome. (b) Time profile of siRNA concentration in the cytoplasm. (c) Time profile of inhibitory effect. (d) Time profile of cell viability. ........................................... 85
Figure 4.9: Trade-off between the total inhibitory effect and the bolus injection. Increasing the infusion from a low dose (1 nM) of siRNA therapeutics to a high dose (500 nM) decreases the total inhibitory effect. ................................................. 86

Figure 4.10: Comparison of the GAMS OCFE/ANN/RK4 simulation results for siRNA delivery over 48-hour transfection.................................................. 87

Figure 4.11: Trade-off between efficacy and toxicity. A relaxation on $Cv^{LO}$ results in a decrease in the minimum total inhibitory effect............................................. 88

Figure 4.12: siRNA delivery optimal control results and the pharmacodynamic responses to siRNA infusion over 48 hr transfection for different lower bounds on cell viability when practical limitations of gene delivery devices are not imposed. (a) Time profile of optimal siRNA infusion. (b) Time profile of siRNA concentration in the cytoplasm. (c) Time profile of inhibitory effect. (d) Time profile of cell viability. ......................................................... 89

Figure 4.13: siRNA delivery optimal control results and the pharmacodynamic responses to siRNA infusion over 48 hr transfection when practical limitations of gene delivery devices are imposed. (a) Time profile of optimal siRNA infusion. (b) Time profile of siRNA concentration in the cytoplasm. (c) Time profile of inhibitory effect. (d) Time profile of cell viability. ......................................................... 90

Figure 4.14: siRNA delivery optimal control results and the pharmacodynamic responses to siRNA infusion over 48 hr transfection when practical limitations of infusion devices are imposed. (a) Time profile of optimal siRNA infusion. (b) Time profile of siRNA concentration in the cytoplasm. (c) Time profile of inhibitory effect. (d) Time profile of cell viability. ......................................................... 91

Figure 4.15: Optimal control results and the pharmacodynamic responses to siRNA infusion over different transfection time periods to study the effect of uncertainty in cell division time when practical limitations are not imposed and $q$ is unconstrained. (a) Time profiles of optimal siRNA infusion. (b) siRNA concentration-time profiles in the cytoplasm. (c) Time profiles of inhibitory effect. (d) Time profiles of cell viability. ......................................................... 93

Figure 4.16: Optimal control results and the pharmacodynamic responses to siRNA infusion over different transfection time periods to study the effect of uncertainty in cell division time when practical limitations are imposed and $q$ is constrained. (a) Time profiles of optimal siRNA infusion. (b) siRNA concentration-time profiles in the cytoplasm. (c) Time profiles of inhibitory effect. (d) Time profiles of cell viability. ........................................................................... 94

Figure 4.17: Optimal control results and the pharmacodynamic responses to siRNA infusion over different transfection time periods to study the effect of uncertainty in cell doubling time when practical limitations are imposed and $q$ is constrained. (a) Time profiles of optimal siRNA infusion. (b) siRNA concentration-time profiles in the cytoplasm. (c) Time profiles of inhibitory effect. (d) Time profiles of cell viability. ........................................................................... 95
**Figure 5.1:** Schematic representation illustrating the relationship between kinetics and dynamics of the vector when considering the pharmacological response (plasma FIX coagulation activity level). .......................................................... 103

**Figure 5.2:** Schematic representation illustrating the relationship between kinetics and dynamics of the vector when considering the toxicological response (ALT level). .......................................................................................... 104

**Figure 5.3:** Estimated PK/PD parameters across different patients .............................. 111

**Figure 5.4:** Pharmacokinetic Analysis, individually for each patient – Comparison of the PK model predictions (using an absolute objective function) with the clinical data. ........................................................................................................ 113

**Figure 5.5:** Pharmacokinetic Analysis, individually for each patient – Comparison of the PK model predictions (using a scaled objective function) with the clinical data. ........................................................................................................ 114

**Figure 5.6:** Pharmacodynamic Analysis, individually for each patient – Comparison of the PD model predictions (using an absolute objective function) with the clinical data. ........................................................................................................ 115

**Figure 5.7:** Pharmacokinetic Analysis, for all patients simultaneously – Comparison of the PK model predictions (using an absolute objective function) with the clinical data. ........................................................................................................ 116

**Figure 5.8:** Pharmacokinetic Analysis, for all patients simultaneously – Comparison of the PK model predictions (using a scaled objective function) with the clinical data. ........................................................................................................ 117

**Figure 5.9:** Pharmacodynamic Analysis, for all patients simultaneously – Comparison of the PD model predictions (using an absolute objective function) with the clinical data. ........................................................................................................ 118

**Figure 5.10:** Linear regression curve between the dose administered and the initial vector concentration in plasma. .......................................................................................................................... 120

**Figure 5.11:** Population pharmacokinetic and pharmacodynamic analysis over a period of 30 days for different initial bolus doses .................................................................................. 121

**Figure 5.12:** Population pharmacokinetic and pharmacodynamic analysis over a period of 60 days for different initial bolus doses .................................................................................. 121

**Figure 5.13:** Population pharmacokinetic and pharmacodynamic analysis over a period of 90 days for different initial bolus doses .................................................................................. 122

**Figure 5.14:** Population pharmacokinetic and pharmacodynamic analysis over a period of 3 years for different initial bolus doses .................................................................................. 122

**Figure 6.1:** Compartmental and spatially distributed system with three compartments. A simple model adapted from Chaudhry (2012) ........................................................................ 127
Figure 6.2: An Artificial Neural Network (ANN) with \( m \) inputs, one hidden layer, \( h \) nodes in the hidden layer and one linear output.................. 131

Figure 6.3: Accuracy of the computed solutions corresponding to problem 1 at the training points. The parameter estimation problem was formulated and solved in GAMS using uniform grid (navy points) and non-uniform grid (red points). The ANN-based model predictions are validated by comparisons with simulation carried out in gPROMS (blue surface). ................................................................. 136

Figure 6.4: Accuracy of the computed solutions corresponding to problem 2 at the training points by comparing the model predictions against the analytical solution (blue surface). The parameter estimation problem was formulated and solved in GAMS using uniform grid (navy points) and non-uniform grid (red points)................................................................. 138

Figure 6.5: Accuracy of the computed solutions corresponding to problem 3 at the training points by comparing the model predictions against the analytical solution (blue surface). The parameter estimation problem was formulated and solved in GAMS using uniform grid (navy points) and non-uniform grid (red points)....................................................................... 140

Figure 6.6: Accuracy of the computed solutions corresponding to problem 4 at the training points by comparing the model predictions against the analytical solution (blue surface). The parameter estimation problem was formulated and solved in GAMS using uniform grid (navy points) and non-uniform grid (red points)....................................................................... 141

Figure 6.7: The star-shaped domain (171 points) and the boundary points (60 points) corresponding to Problem 5. ................................................................. 142

Figure 6.8: Accuracy of the computed solution corresponding to problem 5 at the training points by comparing the model predictions against the analytical solution................................................................. 143
List of Tables

Table 4.1: A summary of the parameter estimation problem. .......................... 96

Table 5.1: Key characteristics of the patients at baseline, according to vector dose. Adapted from Nathwani et al. (2014) ................................................................. 105

Table 5.2: Model parameters and state variables of the PK/PD model................. 106

Table 5.3: Estimated PK/PD model parameters, individually for each patient........ 110

Table 5.4: Estimated PK/PD model parameters, for all patients simultaneously.... 110

Table 5.5: Computational demand for the individual modelling approach.......... 119

Table 5.6: Computational demand for the population modelling approach......... 119

Table 6.1: Example problems 1 – 5.................................................................... 144
Abbreviations

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAV</td>
<td>Adeno-associated Virus</td>
</tr>
<tr>
<td>AE</td>
<td>Algebraic Equation</td>
</tr>
<tr>
<td>ALT</td>
<td>Alanine aminotransferase</td>
</tr>
<tr>
<td>ANN</td>
<td>Artificial Neural Network</td>
</tr>
<tr>
<td>AUC</td>
<td>Area Under the Curve</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary DNA</td>
</tr>
<tr>
<td>CNS</td>
<td>Central Nervous System</td>
</tr>
<tr>
<td>CPU</td>
<td>Central Processing Unit</td>
</tr>
<tr>
<td>DAE</td>
<td>Differential Algebraic Equation</td>
</tr>
<tr>
<td>DDE</td>
<td>Delay Differential Equation</td>
</tr>
<tr>
<td>DE</td>
<td>Differential Equation</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>DO</td>
<td>Dynamic Optimisation</td>
</tr>
<tr>
<td>DPS</td>
<td>Distributed Parameter System</td>
</tr>
<tr>
<td>dsRNA</td>
<td>Double-Stranded RNA</td>
</tr>
<tr>
<td>FDM</td>
<td>Finite Difference Method</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>GAMS</td>
<td>General Algebraic Modelling System</td>
</tr>
<tr>
<td>gPROMS</td>
<td>general Process Modelling System</td>
</tr>
<tr>
<td>GT</td>
<td>Gene Therapy</td>
</tr>
<tr>
<td>HB</td>
<td>Haemophilia B</td>
</tr>
<tr>
<td>LS</td>
<td>Least Squares</td>
</tr>
<tr>
<td>MIMO</td>
<td>Multi-Input–Multi-Output</td>
</tr>
<tr>
<td>ML</td>
<td>Maximum Likelihood</td>
</tr>
<tr>
<td>MOL</td>
<td>Method of Lines</td>
</tr>
<tr>
<td>MPC</td>
<td>Model Predictive Control</td>
</tr>
<tr>
<td>mRNA</td>
<td>Messenger RNA</td>
</tr>
<tr>
<td>NCA</td>
<td>Non-Compartmental Analysis</td>
</tr>
<tr>
<td>NLP</td>
<td>Nonlinear Programming</td>
</tr>
<tr>
<td>OCFE</td>
<td>Orthogonal Collocation on Finite Elements</td>
</tr>
<tr>
<td>OCP</td>
<td>Optimal Control Problem</td>
</tr>
<tr>
<td>ODE</td>
<td>Ordinary Differential Equation</td>
</tr>
<tr>
<td>PBPK</td>
<td>Physiologically Based Pharmacokinetic</td>
</tr>
<tr>
<td>PD</td>
<td>Pharmacodynamics</td>
</tr>
<tr>
<td>PDE</td>
<td>Partial Differential Equation</td>
</tr>
<tr>
<td>PE</td>
<td>Parameter Estimation</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------------------------</td>
</tr>
<tr>
<td>PK</td>
<td>Pharmacokinetic</td>
</tr>
<tr>
<td>PK/PD</td>
<td>Pharmacokinetic/Pharmacodynamic</td>
</tr>
<tr>
<td>PRR</td>
<td>Pattern Recognition Receptor</td>
</tr>
<tr>
<td>RISC</td>
<td>RNA-Induced Silencing Complex</td>
</tr>
<tr>
<td>RK</td>
<td>Runge–Kutta</td>
</tr>
<tr>
<td>RK4</td>
<td>4th order Runge–Kutta</td>
</tr>
<tr>
<td>RNA</td>
<td>Ribonucleic Acid</td>
</tr>
<tr>
<td>RNAi</td>
<td>RNA Interference</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
</tr>
<tr>
<td>SDE</td>
<td>Stochastic Differential Equation</td>
</tr>
<tr>
<td>SG</td>
<td>Stochastic Gradient</td>
</tr>
<tr>
<td>siRNA</td>
<td>Small Interfering RNA</td>
</tr>
<tr>
<td>SNOPT</td>
<td>Sparse Nonlinear Optimiser</td>
</tr>
<tr>
<td>TLR</td>
<td>Toll-like Receptor</td>
</tr>
</tbody>
</table>
Chapter 1.

1 INTRODUCTION

1.1 Background and Motivation

Gene therapy (GT) is a promising technique for treating a wide range of serious incurable diseases, such as genetic disorders and cancer. Gene therapy is broadly defined as a strategy aimed at modifying the genetic makeup of a human cell by using functional genes as a medicine for therapeutic benefit (Ibraheem et al., 2014; Naldini, 2015). A major challenge currently facing the clinical translation of this technology is to ensure that the new genetic information reaches its target inside the cell to drive proficient expression of the therapeutic gene with minimum undesirable interaction (Nam et al., 2009). Therefore, the key to success of gene-based therapy is to develop an optimal gene delivery system for safe and efficient in vivo gene transfer, allowing the transition of discoveries to the clinical arena. A large body of
Chapter 1

research work has evaluated a variety of gene transfer systems or vectors to make the process more efficient and safe (Riley and Vermerris, 2017; Yin et al., 2017). Advances in the development of gene delivery vectors have led to significant clinical success for many genetic conditions, such as haemophilia, blindness, immunodeficiency syndromes, and cancer (Kay, 2011). However, there is much scope for improving gene transfer device systems to help aid in many different issues faced currently in this field. Despite the positive outcomes that have been reported from gene therapy trials for a wide range of genetic diseases, the transition to the clinical phase revealed a number of challenges associated with undesirable responses that limits the application of this therapeutic tool. A novel gene delivery technology based on a multidisciplinary approach involving mathematical analysis, control theory, biological science, and engineering technology, can play a crucial role in providing a platform to help evaluate the translation of developments in the area of gene therapy into clinical practice. While a number of computational works provided insights into the process of gene delivery, most modelling works in literature only partially describe the process and therefore, biological effects, such as toxicity of vectors and cell division events, have largely been ignored. A lot of research is currently ongoing on the design of gene therapy vectors (Kumar et al., 2016); however, no consideration has been given to the development of a comprehensive approach that integrates all the key pharmacological issues into a holistic framework that is applicable for in vivo conditions. The lack of a systematic framework to optimise gene transfer with acceptable safety and efficiency, while still taking into account different practical constraints and variability, revealed the need for developing mathematical models and control algorithms for gene delivery systems.

Motivated by various challenges in in vivo gene therapy, an innovative approach based on the optimal control strategy will be presented to optimise the process of gene delivery in order to demonstrate that the theory of control can be applied to gene delivery systems with relative success. The first essential step towards developing such control strategy is the derivation of mathematical models that adequately describe the behaviour of the system. One of the main challenges during the modelling process is to find a balance between an over-simplified model and a complex model, each with its own set of trade-offs. Although the complex mathematical models provide detailed and extensive analysis of the biological systems, the use of these models in a controller would be limited owing to their size.
Chapter 1

(Parker and Doyle, 2001). Detailed models are likely to contain a large number of parameters that are difficult to be estimated independently, mainly because of the lack of proper experimental data, which is a key ingredient for control applications in biological systems. Therefore, data availability and the objective of model development play a key role over the course of model assembly.

One of the main purposes of this research study is to develop a multi-objective model-based controller algorithm for gene delivery systems to account for the pharmacological responses (intended therapeutic effects of genetic materials) and toxicological responses (undesired or adverse effects of genetic materials) as well as the variability in cell division time so as to optimise the process of gene transfer by computing the optimal gene infusion rates. Modelling and control of biological systems is a challenging task and its complexity increases by taking into account the effect of possible uncertainties and various constraints as well as the inclusion of toxicological responses. Thus, an important factor that must be considered in the modelling process is the size of the mathematical model describing the system’s behaviour. The model should be of a reasonable size to enable the design of a controller for the optimal delivery of therapeutic genes, but still detailed enough to capture the systems dynamics, reproduce the complex pharmacokinetic and pharmacodynamic effects of the transgene, and allow insights and understanding of the system.

The present study focuses on the development of a pharmacokinetic (PK) model involving a system of ordinary differential equations (ODEs) to describe the transport and concentration aspects; and a pharmacodynamic (PD) model based on the Hill equation to describe the pharmacological and toxicological effects of the therapeutic molecules. The integrated PK/PD model is developed based on the available experimental data, which is then used for an optimal control framework that is set up to optimise the gene delivery process, making it applicable for in vivo conditions.

The methodology presented in this work can be extended to incorporate the spatial distribution of the genetic materials resulting in a system of reaction-diffusion partial differential equations (PDEs) in a complex geometrical domain. To this purpose, an efficient and reliable method for solving parameter estimation (PE) problems is required to account for domains with irregular boundaries. Parameter estimation is the key to the development of high-fidelity mathematical models and process control.
Therefore, a theoretical work was performed on the development of a novel meshless framework for estimating the unknown model parameters in a system of partial differential equations using simultaneous optimisation and solution strategy based on the artificial neural network (ANN) approximations.

1.2 Research Objectives

This thesis will highlight the various issues faced in modelling the gene delivery problem, and will focus on the development of an innovative approach based on the optimal control strategy, with the aim of optimising the process of gene delivery.

A flowchart of a typical solution methodology for modelling and control of gene delivery systems is shown in Figure 1.1.

![Flowchart](image)

**Figure 1.1:** Flowchart illustrating the multi-objective model-based controller algorithm for gene delivery systems.
Chapter 1

The model will be developed based on the pharmacokinetic and pharmacodynamic data from literature, and the unknown model parameters will be estimated such that the model predictions being in a good agreement with the experimental data. The model will then be validated using experimental data before being used in an optimal control framework. The reliability of the model will be examined by performing simulation studies using the estimated parameters to determine how well the model can describe the gene delivery process. Then, the gene delivery optimal control problem will be formulated and solved subject to the system models and a set of constraints in order to compute an optimal infusion rate. If the solver fails to converge, the algorithm iterates until it converges.

The main contributions of the thesis are as follows:

1.2.1 Optimal Model-Based Control of Non-Viral siRNA Delivery
RNA interference (RNAi) therapeutics, such as small interfering RNA (siRNA), has become a powerful tool for the post-transcriptional knockdown of defective genes in mammalian cells with the aim of treating severe diseases, such as viral infection and cancer. The aim of this contribution, which will be presented in Chapter 4, is to develop an integrated pharmacokinetic/pharmacodynamic (PK/PD) model-based control algorithm for non-viral siRNA delivery, taking into account the main multi-objective optimisation issues, such as efficacy and toxicity. This chapter will also focus on addressing the variability challenge inherent in the cell division cycle to investigate the effect of possible uncertainties in cell doubling time by incorporating the time constraint into the optimisation problem. An integrated computational framework will be developed that is capable of modelling simultaneously intracellular trafficking of genetic materials and biological effects in order to achieve maximum therapeutic efficacy while minimising toxicity by optimising the dosage schedule of the therapeutic siRNAs, which is applicable for in vivo conditions.

1.2.2 Mathematical Modelling of Gene Delivery in Patients with Haemophilia B
Haemophilia B (HB) is a bleeding disorder resulting from a deficiency of coagulation factor IX (FIX), which may be due to a liver disease or to an inherited defect. In Chapter 5, an integrated pharmacokinetic/pharmacodynamic (PK/PD) model will be developed based on the available clinical data for three patients with severe HB. The
objective is to propose a modelling framework that is capable of predicting the physiological response of a patient affected by type B haemophilia to a dose of vector. The individual PK/PD parameters for each patient will be obtained by solving a parameter estimation problem using the analytical solution and the model will then be validated by carrying out a number of dynamic simulations. The results show a good approximation of the pharmacokinetics and pharmacodynamics of the vector. A simultaneous parameter estimation for all patients will also be performed for each of the PK/PD parameters, which will then be used for initial dose selection.

1.2.3 Parameter Estimation of Partial Differential Equations using Artificial Neural Network

In the work, which will be presented in Chapter 6, an optimisation-based approach will be adopted to formulate and solve a novel meshless parameter estimation framework for a system of partial differential equations using the artificial neural network formulation to account for non-uniform arbitrary domains. The performance of the developed methodology is evaluated extensively on different numerical example problems, demonstrating that the ANN-based approach is very efficient by providing accurate solutions in reasonable computing times. The encouraging results obtained from this work will pave the path for further developments in modelling the gene delivery problem by taking into consideration the cellular architectures to investigate the effect of cellular geometry on the diffusion of genetic materials that needs to be handled by using partial differential equations. This part of the thesis can serve as a starting point to develop a new class of PK/PD models to include both the reaction and diffusion mechanisms affording a more realistic representation.

1.3 Thesis Structure

The structure of the thesis is organised as follows:

Chapter 2, “Review of Gene Delivery Systems”, presents a review of the literature on gene therapy and its current trends. This chapter also introduces the pharmacokinetic/pharmacodynamic modelling approach for biological systems, followed by an overview of previous computational studies on gene delivery systems.

Chapter 3, “Review of Dynamic Simulation and Optimisation”, introduces the mathematical concepts of the optimal control theory, dynamic simulation and
Chapter 1

numerical approximations. A literature review of parameter estimation for PDE models will also be presented.

Chapter 4, “Optimal Model-Based Control of Non-Viral siRNA Delivery”, details a novel framework based on an integrated PK/PD model-based optimal control approach to compute the optimal infusion rates of siRNA therapeutics in the presence of different practical constraints.


Chapter 7, “Conclusions and Future Work”, provides a summary of the thesis, highlights the key contributions, and outlines possible areas of further research.
2 REVIEW OF GENE DELIVERY SYSTEMS

2.1 Introduction

Gene-based therapy has shown remarkable clinical therapeutic efficacy and an excellent safety record for the treatment of various severe inherited diseases. One of the main driving forces behind these advances is the improved vector designs, resulting in a safe delivery of therapeutic genetic materials to specific cells of a patient (Naldini, 2015). However, there is significant room for further improvement in this field to reach therapeutically relevant levels of genetically modified cells. Mathematical modelling and control technologies with the aim of optimising the process of gene delivery can contribute to the revitalization of gene therapy.
Chapter 2

Model-based control theory for drug delivery systems is a well-established field to help understand and predict the mechanisms of drug action and make control-relevant choices (Parker and Doyle, 2001). In this context, the aim of this thesis is to develop an optimal model-based control algorithm for gene delivery systems. This is achieved by developing mathematical models to describe the time course of concentration of therapeutic genes in different compartments and the effect of genetic materials on the body. Through the modelling of the process, optimal delivery profiles can be obtained to maximise the efficacy of gene therapy while minimising toxicity effects.

This chapter begins with an introduction to gene therapy and delivery systems, followed by an overview of biological responses. The concept of pharmacokinetic/pharmacodynamic (PK/PD) modelling will be introduced in Section 2.5. In the final part of the chapter, a review of previous works on model-based gene delivery systems will be presented, in which the previously unaddressed challenges which this thesis will seek to tackle will be identified.

It should be noted that an in-depth review of all nucleotide-based therapies and vehicles that have been or continue to be investigated is outside the scope of this chapter. Only properties of selected nucleic acid molecules and their carriers, which have been used for modelling purposes in this work, will be outlined here.

2.2 Gene Therapy and its Current Trends

Gene therapy (GT) is a promising therapeutic technology (Friedmann, 1996) having the potential to treat diseases at their genetic roots by replacing or counteracting a defective gene within a patient’s cells which are adversely affected by the condition. This therapeutic strategy uses genes as a medicine to provide treatments for diseases that previously were classified as untreatable, and cure those that are treatable but not curable with conventional medications (Kumar et al., 2016).

Over the past decades, gene-based therapy has fascinated scientists, and its clinical application for treating a wide range of diseases has been extensively investigated (Ginn et al., 2013). A first step in this direction was conceived for the treatment of hereditary single-gene defects (Kay and Woo, 1994). Afterwards, several acquired diseases, such as cancer (Lowenstein, 1997; Rochlitz, 2001), cardiovascular diseases (Isner, 2002), acquired immunodeficiency syndrome (AIDS) (Yu et al.,
Chapter 2

1994), infectious diseases (Bunnell and Morgan, 1998), and neurodegenerative disorders (Baekelandt et al., 2000) have been the subject of numerous gene-therapy research. In a landmark study by Nathwani and colleagues, the potential role of gene therapy has been demonstrated in patients with severe haemophilia B (HB) showing the signs of a cure for inherited disorders such as HB (Nathwani et al., 2014).

Theoretically, gene-based therapy is a simple therapeutic approach depending on either correcting a malfunctioning gene by gene editing, or transferring a therapeutic nucleic acid in order to express the required protein (Zhang et al., 2004). However, as simple as the concept sounds, success in clinical trials has been limited due to the complex extracellular and intracellular barriers. The target sites for therapeutic nucleic acid molecules are typically inside the cells, in the cytoplasm or the nucleus (Khalil et al., 2006). An efficient gene transfer must overcome numerous cellular and tissue barriers in order to deliver exogenous genes into the target cells to drive proficient expression of therapeutic molecules, with the eventual goal of altering endogenous gene expression without disrupting essential regulatory mechanisms (Naldini, 2015).

Clinical gene therapies are based on either in vivo or ex vivo gene delivery. In vivo gene therapy involves direct administration of therapeutic genes into a patient. However, in ex vivo gene therapy, the patient’s cells are transduced by therapeutic genes in culture, and the gene-corrected cells are then transplanted back to the patient (Kumar et al., 2016). The optimal model-based control algorithm proposed in this work aims at in vivo gene delivery in order to optimise the process by computing continuous optimal infusion rates.

Researchers in fields ranging from nanomedicine to gene therapy have investigated a broad range of nucleotide-based therapies including DNA (Friedmann, 1992), small interfering RNA (Dorsett and Tuschl, 2004), antisense oligonucleotides (Dias and Stein, 2002), microRNA (Bartel, 2009), aptamers (Lee et al., 2006) and messenger RNA (DeRosa et al., 2016), to be delivered into target cells with the aim of treating severe diseases. This thesis has predominantly focused on the siRNA and DNA used for modelling and control purposes. Traditionally, gene therapy has been primarily defined as a process by which an exogenous DNA is transferred and introduced to host cells for expressing therapeutic transgenes. The process of introducing DNA into the cells for the purpose of gene expression is called
transfection or DNA delivery (Heiser, 2004). However, naked DNA molecules are large and fragile, cannot cross the cell membrane, and go through rapid degradation upon systemic administration. Therefore, delivery carriers should be used to facilitate DNA transfer (Song et al., 2017). The process of DNA delivery begins with the DNA condensation and complexation, the introduction of DNA into the systemic circulation, targeted gene transfer to specific cells followed by cellular uptake (endocytic or non-endocytic), endosomal release, nuclear targeting/entry, and unpacking of the DNA/carrier complexes before the final step of translation which involves the production of a functional protein (Luo and Saltzman, 2000; Shi et al., 2017). Although DNA-based gene therapy has shown considerable therapeutic potential, the engagement of other types of nucleic acids are also contributing to the revitalization of gene therapy (Scholz and Wagner, 2012). RNA interference (RNAi) is one of the most powerful tools that emerged in 1998, and has been developed as a novel therapeutic approach to target specific genes with the aim of treating diseases. RNAi is defined as a process of specific post-transcriptional gene silencing mediated by small RNAs (Tang et al., 2016). Fire, Mello and co-workers discovered the ability of double-stranded RNA (dsRNA) molecules to silence gene expression in animal cells (Fire et al., 1998). In 2001, Tuschl and colleagues published their work and demonstrated that synthetic small interfering RNA (siRNA) transfected into mammalian cell lines could achieve sequence-specific gene knockdown in adult mammalian cells (Elbashir et al., 2001). Shortly thereafter, McCaffrey’s group achieved the first successful application of siRNA for gene silencing in adult mice for a hepatitis C target (McCaffrey et al., 2002). siRNA is a potent tool in RNAi-based gene therapy for the treatment of a wide range of diseases that are associated with undesirable gene expression. Since its discovery, the strategy of using synthetic siRNA as a therapeutic agent has been extensively investigated for the post-transcriptional knockdown of defective genes in mammalian cells to treat diseases, such as viral infection and cancer (Whitehead et al., 2009).

2.3 Gene Delivery Systems

The key to a successful gene therapy is the development of safe and efficient gene delivery vehicles, which also known as vectors, in order to deliver the therapeutic genes to the target tissue without degradation (Ibraheem et al., 2014). Given the diversity of disease targets, which are potentially amenable to gene delivery, as well
as the different range of nucleotide-based therapeutics, it has been concluded that there can be no single vector that is suitable for all GT applications. Therefore, considerable progress has been made in developing numerous vectors to ferry the therapeutic genes into the target tissues, and several of which have been employed in clinical trials of *in vivo* gene transfer. More information of this topic can be found in the Journal of Gene Medicine Clinical Trial Database (Wiley Database), which contains information on human gene-transfer clinical trials worldwide.

Gene therapy vector systems, which have successfully demonstrated the transfer of exogenous genes *in vivo*, are broadly classified into two major groups: viral and non-viral vectors (Liu and Huang, 2002). Note that the scope of this section is limited to review the viral vectors based on adeno-associated virus, and non-viral vectors based on cationic lipids, which were employed in gene delivery applications whose data were used for modelling and control purposes in the current work.

Viral delivery systems utilize viral vectors to encapsulate therapeutic genes to facilitate efficient delivery (Yin et al., 2017). In general, the most commonly used classes of virus-based vector systems in gene therapy can be broadly categorised into two groups according to whether their genomes integrate into host cellular chromatin, such as oncoretrovirus and lentivirus vectors; or persist predominantly as extrachromosomal episomes in the cell nucleus, such as adeno-associated viruses (AAVs), adenoviruses and herpes viruses (Thomas et al., 2003). To use a virus as a therapeutic tool to ferry a gene into target sites, it must be modified by genetic engineering (Ibraheem et al., 2014). Among gene transfer vectors of viral origin, adeno-associated virus (AAV) vector-mediated gene transfer has progressed rapidly over the past decade, making remarkable strides for the treatment of liver-based diseases as demonstrated in a number of clinical trials, which are currently planned and ongoing (Kattenhorn et al., 2016; Nathwani et al., 2017). AAV vectors belong to the parvovirus family, which is dated back to the 1960’s (Hoggan et al., 1966; Rose et al., 1966). An AAV viral vector consists of a protein coat or viral capsid, a vector DNA genome, and the therapeutic transgene product that the DNA encodes (Mingozzi and High, 2013). A wide array of serotypes (i.e., AAV1 through AAV9) and differences in the vector capsid enables the targeting of a variety of cell types and tissues (Kumar et al., 2016). AAV-mediated gene transfer has been used in the central nervous system (CNS) (Kaplitt et al., 2007; Leone et al., 2012), the retina
(Maguire et al., 2008), the liver (Manno et al., 2006; Nathwani et al., 2014), and cardiac muscle (Greenberg et al., 2016). AAV-based gene transfer protocols have a number of attractive features including high transfection efficiency and prolonged expression of a therapeutic transgene (Ibraheem et al., 2014). AAV vectors have shown remarkable success in both pre-clinical and clinical studies of in vivo gene-based therapy for genetic diseases, such as haemophilia B, by demonstrating a long-term expression of therapeutic levels of coagulation factor IX (Nathwani et al., 2014). Despite a strong record of success, the application of this technology in the clinical phase revealed a range of challenges, mainly in the form of a human-specific immune response against the vector (High and Anguela, 2016).

Non-viral vectors, on the other hand, have provided an alternative tool following a failure of clinical trial by viral gene transfer using adenovirus vector in 1999, which resulted in a patient death (Ferber, 2001). Non-viral delivery technologies mediate the entry of therapeutic genes into the target cells either by physical methods or chemical methods. Physical gene delivery methods, such as gene gun and electroporation, allow to create transient holes on the cell membrane to enable the free entry of therapeutic molecules. However, chemical methods are carrier-based methods using cationic lipids or cationic polymers as gene vectors to package and condense nucleic acid molecules into positively charged particles, which could be easily uptaken by cells via endocytosis (Wang et al., 2013). Non-viral vectors are potentially less immunogenic, less toxic, able to be modified, and relatively easy to produce on a large scale (Jiang et al., 2012). However, they present their own set of delivery challenges. Despite considerable progress in this field, the application of delivery vectors based on non-viral materials in human therapy remains a major challenge owing to low transfection efficiency and poor transgene expression (Wang et al., 2013; Yin et al., 2017). Significant efforts have been made to improve non-viral delivery carriers and a number of recent studies have pointed to the design and structure of various types of non-viral systems (reviewed in detail by: Videira et al., 2014; Williford et al., 2014; Yin et al., 2014). In this work, a type of cationic lipid-based nanocarrier (NC), SPANosomes (SP) formulation, which was developed and evaluated by Zhou et al. (2012), was investigated for the development of model-based optimal control algorithm. Cationic lipids were introduced for the first time in 1987 by Felgner and colleagues in order to facilitate the delivery of DNA into target cells. Since then, a large number of cationic lipid derivatives have been synthesized
and used in nucleic acid delivery (Miller, 1998; Heyes et al., 2005; Zhang et al., 2007; Akinc et al., 2008; Mintzer and Simanek, 2009; Lu et al., 2009; Love et al., 2010; Semple et al., 2010). Potential complications of chemical gene delivery systems will be reviewed in the next section.

2.4 Biological Responses

Further understanding of the intracellular transport and biological effects involved in cellular and intracellular delivery of nucleic acid materials is required to produce clinical applications of gene therapy. In a RNAi-based gene therapy, the desired pharmacological effect of a siRNA therapeutic is to silence specific genes via RNAi machinery. Once a siRNA molecule is delivered into a cell, it is loaded onto the RNA-induced silencing complex (RISC), which then undergoes cleavage by a protein within RISC, followed by the target mRNA recognition and degradation resulting in gene silencing (Whitehead et al., 2009). Due to the unfavourable physicochemical properties of siRNA molecules, such as large, unstable, hydrophilic and anionic structure, naked siRNAs can be easily degraded by biological components. Therefore, carriers should be used for escorting the siRNA molecules across cellular barriers (Wang et al., 2010).

Several biological barriers and interactions between nanocarriers and biomolecules can restrict efficient siRNA delivery (Wang et al., 2013). Regardless of the route of administration, the final destination of siRNA cargo is the cytoplasm of the target cell. After intravenous injection, therapeutic molecules are distributed to organs via the blood circulation. Within an organ, therapeutic materials leave the blood vessels to enter the interstitial compartment. The interstitial fluid is part of the extracellular fluid that surrounds all cells in the body. Once a siRNA/NC complex is bound to the target cell membrane, it is internalized into the cell by endocytosis, which is the major mode of internalization. Endocytosis is a process in which the siRNA/NC being encapsulated in endocytic vesicles, formed by internalization of the cell membrane, that fuse with endosomes. siRNA must be capable of escaping the endosomes to be released from its carrier to the cytoplasm so as to be loaded onto RISC (Wang et al., 2010). Coating of carriers with ligands facilitates cellular uptake of siRNA by the specific ligand-receptor binding interactions. Furthermore, the cationic siRNA/NC complex can interact with the negatively charged proteins on the cell membrane through electrostatic interactions, and form an endocytic vesicle that fuse with an
Chapter 2

early endosome maturing into a late endosome and then fuse with a lysosome. One of the major impediments for non-viral nanocarriers is to escape from the endosome/lysosome compartment to avoid enzymatic degradation (Dykxhoorn et al., 2006; Kanasty et al., 2012; Wang et al., 2010).

Early endosomes mature into the late endosomes with the pH value around 5, then fuse with lysosomes. In order to achieve efficient delivery of siRNA therapeutics, it is expected that endosomal escape occurs before siRNA molecules are transferred to the low-pH and enzyme-rich environment of the lysosomes aiming at degradation of the therapeutic cargo. The pH value in early endosomes is around 6, which is lower than that of the circulation system, allowing the NCs to release their cargo after entering endosomes in a cell due to the pH decrease. However, acidifying endosomal environment may promote NC dissolution in the cell leading to the distribution of toxic ions, and so cell death (Kanasty et al., 2012; Lv et al., 2006; Nel et al., 2009; Wang et al., 2010; Zhou et al., 2013).

siRNA-loaded nanocarriers (NCs) can provide excellent platforms for therapeutic siRNA molecules to be protected from unintended interactions with biological surfaces and from degradation or metabolism. However, the interactions between NCs and cells can cause deleterious effects. Therefore, understanding of the dynamic forces, molecular components, nanomaterial properties, and cellular uptake is of critical importance (Frohlich, 2012; Kim et al., 2009; Nel et al., 2006, 2009).

In order to improve carrier-cell interactions, different features, such as inclusion of cationic materials, have been introduced to the nanocarriers. However, the high complexity of carriers can be counterproductive as each additional feature could imply an extra risk of toxicity. Therefore, development of an effective and safe delivery systems is one of the main engineering challenges of nucleic acid-based therapy (Xue et al., 2014).

According to Nel et al. (2009), the main biophysicochemical effects on the interface between a nanomaterial and biological systems stem from (i) the dynamic interactions, such as hydrophilic and hydrophobic interactions, electrostatic and receptor-ligand binding interactions; (ii) material properties, such as size, surface charge, ligands, hydrophobicity and hydrophilicity; and (iii) modification of the surface properties, including the incorporation of surfactants, polymers and lipids. Specific
and nonspecific binding interactions play an important role in cellular uptake. Among the specific binding forces, ligand-receptor interaction is the most effective specific interaction that allows the material decorated with ligands to interact with complementary molecules or receptors on the cell membrane, resulting in receptor-mediated endocytosis. On the other hand, nonspecific binding forces result from particle surface characteristics, such as surface charge and hydrophobicity. Surface charge plays a key part in the interactions between particles and protein domains or charged phospholipid head groups on cell surfaces (Fleck and Netz, 2004; Nel et al., 2009). The cell membrane consists primarily of lipids and phospholipids; thus, using lipid-based nanocarriers for siRNA delivery systems can facilitate cellular uptake owing to their natural tendency to interact with the cell surface. The SP formulation (SPANosomes), which was reported by Zhou et al. (2012), is composed of cationic lipids and nonionic surfactants. Cationic lipids contribute actively to the lipid-based siRNA formulations through: (i) improving the interaction of the cationic lipid bilayer with the negatively charged siRNA molecules, allowing for higher encapsulation efficiency; and (ii) providing a siRNA-carrier complex with a net positive charge to interact with the negatively charged cell membrane via electrostatic interactions, allowing for efficient cell membrane binding (Chen, 2009; Xue et al., 2014).

The most common method to enhance siRNA delivery is the use of nanocarriers (NCs) (Keshawani et al., 2012). However, from a toxicological perspective, particle size and surface area are two important material characteristics. As the particle size decreases, the surface area increases, and therefore the activity increases as a greater proportion of atoms or molecules are displayed on the surface of the material rather than its interior. This leads to a complicated set of interactions between NCs and the biological systems, increasing the possibility of causing damage to human body at the organ, tissue, and cell levels (Nel et al., 2006; Xue et al., 2014).

Cellular damage can be associated with Reactive Oxygen Species (ROS). The interaction of electron donor or acceptor active sites on the surface of a NC with molecular dioxygen ($O_2$) can catalyse ROS production, leading to toxicological effects. What is notable here is that living organisms can produce ROS as a result of normal cellular metabolism. Having low to moderate concentration of ROS could function in physiological cell processes; however, high concentrations may stimulate the natural antioxidant defenses causing an oxidant imbalance in the cell and
produce adverse modifications to cell components, such as DNA. Therefore, the risk of cell death can be increased (Birben et al., 2012; Nel et al., 2006; Xue et al., 2014).

Delivery vehicles could influence other mechanisms, such as immunostimulation. Innate immune system controls the process of immunological response to non-self molecules, such as nucleic acids and their carriers. The innate immune response is triggered by the stimulation of pattern recognition receptors (PRRs), which are responsible for the recognition of foreign pathogenic patterns. There are two types of siRNA-recognizing PRRs: toll-like receptors (TLRs) and cytoplasmic receptors. Delivering siRNAs into endosomes can stimulate endosomal TLRs to induce an immune response. siRNAs delivered into the cytoplasm can interact with several cytoplasmic receptors that mediate immune responses (Kanasty et al., 2012).

Figure 2.1: Intracellular barriers of siRNA delivery using non-viral delivery systems.

Figure 2.1 shows the intracellular barriers of siRNA delivery using non-viral delivery methods. Naked siRNA shows poor cellular entry due to the unfavourable physicochemical properties. However, using nano-sized carriers for escorting siRNA
molecules can enhance the cellular delivery of therapeutic materials. Specific and nonspecific interactions play key roles in cellular uptake of siRNA/NC complexes. After endocytosis, siRNA must escape from the endosome-lysosome degradation axis in order to be loaded onto the RNA-induced silencing complex (RISC). The journey of siRNA/NC from extracellular matrix to its final destination, cytoplasm, increases the risk of cytotoxicity that is attributed to the production of Reactive Oxygen Species (ROS), release of toxic ions due to the interaction between NCs and biomolecules, stimulation of endosomal toll-like receptors (TLRs), and influx of calcium into the cell (Frohlich, 2012; Kanasty et al., 2012; Nel et al., 2006; Xue et al., 2014).

Although cationic lipids facilitate cellular internalization due to their positive charge, a higher toxicity of such materials has been reported, in comparison with the neutral or negatively charged carriers. Cellular toxicity of cationic lipids could be attributed to activation of TLRs (Kedmi et al., 2010; Soenen et al., 2009) through the interactions of cationic groups with critical enzymes (Lv et al., 2006). Moreover, production of additional ROS is another potential factor that has been reported to contribute to the toxicity of cationic lipids. Positively charged NCs can also cause disruption of cell membrane integrity leading to influx of Ca\textsuperscript{2+} and so cell death (Frohlich, 2012).

This section provided a summary of the complexity of gene delivery and various biological responses, such as toxicity, which significantly affect transfection. Quantitative analysis of intracellular pharmacokinetics and pharmacodynamics is particularly important for developing effective and safe gene delivery systems. However, as most of the biological phenomena are complex and not fully understood, it is difficult to model in detail all the mechanisms due to the complexity of the process and lack of suitable experimental data. Fundamentals of mathematical modelling for gene delivery systems are presented in the next section.

2.5 Modelling of Gene Delivery Systems

The art of successful modelling has relied on a number of factors, as listed below:

I. Proper data which is a key ingredient for control applications in biological systems;

II. Model development and parameter estimation;

III. Analyse output and evaluate reliability.
Chapter 2

This section aims at understanding the field of modelling in gene delivery systems. An overview of the pharmacokinetic and pharmacodynamic modelling is presented in Sections 2.5.1 and 2.5.2, while Section 2.5.3 presents a broad review of modelling approaches in the field of gene delivery, and highlights the main limitations of current methods in this area of study.

2.5.1 Pharmacokinetic Modelling

Pharmacokinetics is the study of absorption, distribution and elimination of a drug in the body, and its origins dating back to the 1950’s when Dost first introduced the term *pharmacokinetics* in 1953 (Dost, 1953). Pharmacokinetic (PK) modelling is a well-established field in terms of understanding the pharmacological phenomena and analysing the mechanisms that underpin biological events in drug/gene delivery processes. While there are different classifications of PK models available in literature, a type of classification of pharmacokinetic models is broadly divided into two general approaches: compartment modelling and non-compartment modelling (Peng and Cheung, 2009). Non-compartment modelling approach, which is also known as non-compartmental analysis (NCA), deals with determining the degree of exposure following administration of a drug such as area under the concentration-time curve (AUC), and the drug’s associated PK parameters such as the peak concentration, peak time, clearance, and elimination half-life (Gabrielsson and Weiner, 2010). Compartment modelling approach studies the time course of drug concentration in a certain class of conceptual units, known as compartments. The organism to which the therapeutic materials are administered is assumed of as a system of interconnected pools, which are referred to as compartments. This class of pharmacokinetic models determines how the drug concentration changes over time (i.e. what the body does to the drug), and two major groups of models which fall under this category include mechanistic models and physiological models (Dahl and Akerud, 2013; Holz and Fahr, 2001). The mechanistic interpretation through mathematical models represent a simple plot of compartment modelling as the compartments in this class of models do not reflect functional entities of the organism. However, physiologically based pharmacokinetic (PBPK) models are much more complex as they include several compartments representing important biological subsystems such as blood, the lymphatic and the central nervous system, tissues, organs, and other body spaces. PBPK models are described by complex
interactions in which each compartment corresponds to an organ which is interconnected by real-life material fluxes such as blood flow and lymph flow (Hall et al., 2012; Holz and Fahr, 2001).

Despite the fact that detailed compartmental model structures provide more realistic representation of a biological system as they account for the drug kinetics, biochemistry, physiology and anatomy, leading to a better predictive performance, the utility of these models in a model-based controller would be limited owing to their size (Parker and Doyle, 2001). In this thesis, the mechanistic compartment modelling approach is applied to describe the pharmacokinetics of nanocarrier-mediated siRNA (Chapter 4) and adeno-associated-viral (AAV) -mediated gene therapy (Chapter 5), and address the mechanisms of gene delivery in vivo by incorporating the infusion rate in the mathematical description.

Mathematical structure of a pharmacokinetic model typically involves a set of ordinary differential equations. The simplest PK model is a one-compartment model representing the systemic circulation (Figure 2.2).

![Figure 2.2: The one-compartment model with first-order elimination.](image)

The relationship between the gene concentration, $C$ (mass / volume), and the rate of change, $dC / dt$, of the concentration in a single compartment may be expressed mathematically with first-order kinetics when the therapeutic gene is administered as a bolus dose as follows:

\[
\frac{dC}{dt} = -\theta \cdot C
\]

where $\theta$ (time$^{-1}$) is the first-order rate constant associated with the elimination process (Gabrielsson and Weiner, 2010). Often, it is insufficient for a patient to have therapeutic effect of a drug over the time span in which a single intravenous bolus dose is active in the body. In some cases, administration of a higher dose might be the solution; however, this may cause undesirable side effects. Therefore, in order to prolong the therapeutic effect of a drug, a continuous infusion may be a reasonable
treatment option, allowing for controlling the flow rates of the drug into the body in *in vivo* conditions, which can be modelled as follows:

\[
\frac{dC}{dt} = -\theta \cdot C + q(t)
\]

where \(q(t)\) (mass/volume . time) is the flow rate of the therapeutic materials to be infused.

Variations in model topology can be explored in the course of model assembly in order to develop a configuration that can accurately capture the available experimental data and adequately describe the transport and concentration aspects.

### 2.5.2 Pharmacodynamic Modelling

Pharmacodynamics (PD) is an area of pharmacology concerned with the pharmacological effects of a drug on a biological system. It can be defined as the study of the relationship of the response to drug exposure, and the time course of the biological effects of therapeutic materials. There are a number of definitions that are crucial to the pharmacodynamic concepts considered in this thesis, such as *efficacy* and *toxicity*. Efficacy can be regarded as the desired response that a drug can produce in the case of a pharmacological effect. However, toxicity is defined as the undesired response that occurs after administration of the drug in the case of a toxicological response (Cutler et al., 1994; Gabrielsson and Weiner, 2010).

Pharmacodynamic models may be broadly grouped into two classes of *direct* and *indirect*. Following infusion, there is a build up of the pharmacological response which is governed by stimulatory and/or inhibitory factors controlling the response. The length of the time frame, which is required to observe the pharmacological response, often determine the category of the PD model (*direct* or *indirect*). A direct response model, which is also referred to as the instantaneous response model, can be assumed when the response is directly driven by the drug concentration. Direct pharmacodynamic models commonly describe the concentration-response relationship. The pharmacological response can be measured and modelled as a function of the drug concentration in the effect site compartment by the sigmoid \(E_{\text{max}}\) model (Equation 2.3), which is a derivation of the Hill function (Gabrielsson and Weiner, 2010). The Hill equation was originally introduced by Hill (1910) to describe the relationship for oxygen-haemoglobin association. Now, it is known as a standard
equation in pharmacology (Goutelle et al., 2008).

\[ E = \frac{E_{\text{max}} \cdot C^\gamma}{EC_{50}^\gamma + C^\gamma} \]

where \( E (\%) \) is the effect, \( E_{\text{max}} (\%) \) describes the maximum effect, \( C (\text{mass} / \text{volume}) \) represents the drug concentration, \( EC_{50} (\text{mass} / \text{volume}) \) is the concentration triggering 50% of the total effect, and \( \gamma \) is the slope of the Hill equation to account for the curvature and does not have a direct biological interpretation.

An indirect response model, which is also referred to as the turnover model, allows for a temporal difference to be accounted for, when the response takes time to develop and the observed effect is not directly related to the plasma concentration of the therapeutic material. Therefore, the PD model must account for the effect of time, which can be added as a variable in the non-steady state analysis. Therefore, indirect response models are typically defined using differential equations. In such PD case, there is a time delay between the observed pharmacological/toxicological response and the plasma concentration of the drug (Gabrielsson and Weiner, 2010).

A compartmentalisation of the body, which may include one-, two-, or multi-organ or tissue systems, is shown in schematic form in Figure 2.3, demonstrating the relationship between kinetics and dynamics of a drug and its journey in the body from the absorption site to the blood and the different steps involved in the distribution, metabolism and elimination of the drug.

Figure 2.3: Schematic representation illustrating the relationship between kinetics and dynamics of a drug. Adapted from Gabrielsson and Weiner (2010).
2.5.3 Mathematical Modelling of Gene Delivery Systems

The application of advanced modelling and control strategies in a gene delivery system allows for optimising the delivery amount, timing, and speed in an effective and safe way. In experimental medicine, computational approaches are valuable surrogates where experiments are impossible or difficult to conduct due to practical limitations or ethical concerns (Cutler et al., 1994). The area of modelling in gene delivery systems has emerged since Ledley and Ledley (1994) developed a multi-compartment and numerical model for studying the kinetics of cellular processes. The authors developed a six-compartment model to simulate the main processes involved in gene delivery and expression. Since then, researchers have made considerable efforts in the development of mathematical and computational models for understanding the cellular transport processes (Schwake et al., 2010; Zhou et al., 2007). Most authors (Banks et al., 2003; Ledley and Ledley, 1994; Varga et al., 2001, 2005) developed mathematical models based on the concepts of mass action kinetics to describe the dynamics of the gene delivery system and investigate the critical steps involved in the process. Although the exact mechanisms of the biological effects and the transfection process are not fully understood, further development of mathematical and computational methodologies has provided insights into the gene delivery process. Such methodologies include quantitative structure–activity relationship (QSAR) modelling strategy (Horobin and Weissig, 2005), stochastic simulations (Dinh et al., 2007), semi-mechanistic model of transgene expression (Berraondo et al., 2009), mechanistic spatiotemporal and stochastic model of DNA delivery (Jandt et al., 2011), and telecommunication model (Martin et al., 2015). Despite advances in this field, no effort has been made to develop a holistic framework that is applicable for in vivo gene therapy, and could provide a model-based decision-making platform taking into account the main multi-objective optimisation issues, which consequently forms the main objective of the thesis.

A literature survey revealed that while a number of research works have been done on the model-based control of drug delivery (Dua et al., 2010), very little thought had been given to the development of control strategies for gene delivery systems (Dua, 2012; Ma and Zhang, 2009). This work will demonstrate a novel framework in the field of modelling and control of gene delivery systems. The proposed modelling
framework follows a compartmentalization approach. Compartmental models (Banks et al., 2003) are amenable for control purposes (Parker and Doyle, 2001), an advantage over detailed models (Dinh et al., 2007). According to Parra-Guillen et al. (2010), pharmacokinetic/pharmacodynamic (PK/PD) modelling approach can be valuable in the optimisation and rational development of non-viral-vector-based gene-therapy. Thus, effective mathematical models would consider both the pharmacokinetics and the pharmacodynamics (Mac Gabhann et al., 2010). To the best of the author’s knowledge, the focus of existing mathematical modelling in many gene delivery studies has so far been mainly on one or the other.

A thorough literature search revealed no appropriate integrated computational framework that is capable of modelling simultaneously trafficking of therapeutic genes as well as the pharmacological and toxicological responses while taking into account the effect of possible uncertainties in cell doubling time. Most modelling works in literature only partially describe the complexity of gene delivery processes. The key limitations of previous computational studies are highlighted in this section and listed below:

(i) Toxicity of vectors was left unaccounted for (Banks et al., 2003; Dinh et al., 2007; Jandt et al., 2011; Varga et al., 2001, 2005);
(ii) Effects of cell division were not incorporated (Banks et al., 2003; Dinh et al., 2007; Varga et al., 2001, 2005);
(iii) Unable to predict transfection for in vivo applications (Banks et al., 2003; Martin et al., 2015);
(iv) Effect of uncertainty in cell doubling time was not addressed (Banks et al., 2003; Dinh et al., 2007; Martin et al, 2015; Varga et al., 2001, 2005).

The literature review showed that while a number of works accounted for the modelling and simulation of gene delivery systems, a comprehensive approach that integrates all the key pharmacological issues into a holistic framework that is applicable for in vivo conditions is still lacking. It will be shown in Chapters 4 and 5, via two case studies, that the integrated modelling framework can be applied to gene delivery systems with relative success. However, before presenting the details of the methodology developed for this purpose, an overview of the dynamic simulation and optimisation will be presented in Chapter 3.
Chapter 2

2.6 Summary

This chapter presented an overview of the gene therapy strategy, gene transfer systems, and biological responses in the process of gene delivery (Sections 2.2-2.4). This was followed by an introduction to the concepts of mathematical modelling in biological systems (Section 2.5). A review of the previous computational approaches in this area of study was then presented in Section 2.5.3. Finally, the unaddressed challenges which a part of the thesis will seek to tackle were identified. The review showed that previous works on modelling of gene delivery systems only partially describe the complexity of the process, therefore the need to develop a comprehensive framework to address the key pharmacological issues was identified.
Chapter 3.

3 REVIEW OF DYNAMIC SIMULATION AND OPTIMISATION

3.1 Introduction

This chapter focuses on the theoretical concepts that are concerned with the simulation techniques, parameter estimation problem and the optimal control theory. One of the main objectives of this chapter is to fundamentally understand the dynamic simulation and optimisation, which is a stepping stone on the pathway to the model-based optimal control of gene delivery systems.

Moreover, one of the novelties of this thesis lies in demonstrating how Artificial Neural Network (ANN) formulation can be applied to parameter estimation of system of Partial Differential Equations (PDEs). The details of this contribution will be
Chapter 3

presented in Chapter 6. Therefore, before proceeding with the development of the parameter estimation problem and the application of ANN-based approximations, it is crucial to have a broad overview of mathematical modelling, numerical approximations, and parameter estimation.

The rest of this chapter is structured as follows. Section 3.2 will present a general introduction to differential equations. The concept of optimal control theory will be introduced in Section 3.3, followed by an overview of solution strategies for dynamic optimisation problems. Numerical approaches for solving dynamic systems will be discussed in Section 3.4. Section 3.5 will focus on the application of optimisation approaches for estimating model parameters in differential equation systems. A review of literature on parameter estimation problems for PDE models will be presented in Section 3.5.2, which highlights potential areas of improvement to which the last part of the thesis (Chapter 6) will seek to contribute to the state-of-the-art. This will be followed by a summary of the present chapter in Section 3.6.

3.2 Overview of Differential Equations

Change is the most critical aspect of many systems in scientific fields, hence differential equations play important roles in modelling dynamic processes. In general, a differential equation (DE) involves the rate of change of a dependant variable, which is a physical quantity such as the plasma concentration in a gene delivery system. Many problems in biology, physics, mathematics, chemistry and engineering can be modelled by using differential equations. Generally, they are classified into different categories (Gershenfeld, 1999):

- Ordinary differential equation (ODE): an ODE is an equation (or system of equations) in which the unknown function (or the dependant variable) is a function of a single independent variable (such as time).
- Partial differential equation (PDE): a PDE is a differential equation written in terms of an unknown function and its partial derivatives with respect to multiple independent variables (such as time and space).
- Differential algebraic equation (DAE): a DAE is an equation involving differential and algebraic terms, which is a generalized form of ODEs.
- Stochastic differential equation (SDE): in a SDE, one or more of the terms are stochastic parameters, result in a solution which is a stochastic process.
• Delay differential equation (DDE): a DDE studies the state of a system not only in its present state, but also at certain times in the past.

Differential equation problems appear frequently in numerous process systems. The focus of this research work is on dynamic models involving a system of ordinary differential equations (ODEs) and partial differential equations (PDEs). The link between differential equations and real-world problems will be studied in the rest of the thesis.

3.3 Optimal Control

Optimal control is a well-established branch of control theory with enormous applications in both science and engineering. The optimal control theory is concerned with obtaining the control signals while simultaneously optimising (i.e., maximising or minimising) some performance criterion subject to the physical constraints. The mathematical formulation of an optimal control problem (OCP) involves three fundamental parts: (i) an optimisation objective function (performance criterion/index) to represent the mathematical description of a phenomenon whose maximum or minimum is sought; (ii) a mathematical formulation of the process system to be controlled; and (iii) a statement of the constraints to determine a search space for the optimisation framework. Optimal control of any process can be either in open-loop or closed-loop form (Kirk, 1970). In the present work, an optimal control framework based on the open-loop policies, which is also referred to as Dynamic Optimisation (DO), was developed for gene delivery dynamic systems since no disturbances were considered over the course of modelling.

The time-continuous optimisation problem can be formulated mathematically in the following form:

$$
\min_{\Psi(t), q(t), \theta} \varphi(\Psi(t), q(t), \theta, t) \quad 3.1
$$

subject to the dynamic model

$$
\frac{d \Psi(t)}{dt} = F_k(\Psi(t), q(t), \theta, t), \quad \Psi(t_0) = \Psi_0 \quad 3.2
$$

and constraints
\[ \phi_{LO} \leq \phi[\Psi(t), q(t), \theta] \leq \phi_{UP} \]

where \( \phi \) is an objective function, \( \Psi(t) \in \mathbb{R}^{n\Psi} \) is the time dependant vector of state variables, \( q(t) \in \mathbb{R}^{nq} \) denotes the time-variant vector of control variables, \( \theta \in \mathbb{R}^{n\theta} \) indicates a vector of static parameters (or time-independent decision variables), and the initial state of the process is denoted by \( t_0 \) (independent time variable).

Solution strategies that can be used to solve the dynamic optimisation (DO) problems are broadly classified into two main frameworks: variational (indirect) approaches and discretisation (direct) approaches (Cervantes and Biegler, 2000). The class of variational or indirect approaches can facilitate requirements for the problems without inequality constraints. For the case in which inequality constraints need to be considered, the indirect approaches are not appropriate as additional conditions for optimality are required (Bryson, 1975; Pontryagin, 1962). In order to deal with constrained problems, direct approaches play important roles since they transform the DO problem into a finite-dimensional nonlinear programming (NLP) problem. Discretisation (direct) approaches can be separated into two categories, sequential methods (control vector parameterization) (Kraft, 1985; Sargent and Sullivan, 1978) and simultaneous approaches (direct transcription) (Biegler, 1984; Tsang et al., 1975). In the sequential strategies, which is also known as partial discretisation, only the control variables are approximated. Although these methods require the solution of small NLP problems, they cannot handle instabilities properly (Biegler, 2007). In contrast with the sequential methods, in the simultaneous approaches or full discretisation, both the control and state variables are discretised in time by hiring an appropriate discretisation scheme (principal numerical approximations will be briefly discussed in the next section). In the current work, the direct-simultaneous approach was chosen for the solution of the OCP since it offers a number of advantages including the treatment of constraints and unstable dynamic systems. Biegler (2007) highlighted the main advantages and characteristics of this approach.

### 3.4 Numerical Methods for Solving Differential Equations

There are many approaches for solving differential equations (DEs), such as Euler’s method, Runge–Kutta (RK) schemes, Finite Difference Method (FDM), predictor-corrector methods, separation of variables, Laplace transforms, shooting methods,
Orthogonal Collocation on Finite Elements (OCFE), and Artificial Neural Network (ANN). In this work, Runge–Kutta, OCFE and ANN methods were adopted as approximation elements for the numerical solution of the DEs. A brief review of these methods is presented in this section.

### 3.4.1 Fourth Order Runge–Kutta (RK4)

The family of Runge–Kutta (RK) discretisation schemes is the most famous of multistage schemes, which are popular workhorses for solving ODE problems. The idea behind this class of ODE integrators is to use transformations and the Butcher tableau to numerically compute the future time-step. A well-known fourth order Runge–Kutta (RK4) scheme is the most widely used time-integration method of all Runge–Kutta solvers, and is conditionally stable.

Mathematically, the method can be expressed as:

\[
\psi_{i+1} = \psi_i + \frac{(k_1 + 2k_2 + 2k_3 + k_4)}{6} \tag{3.4}
\]

where \( k_1, k_2, k_3, \) and \( k_4 \) have the form:

\[
k_1 = h f(\psi_i, t_i) \tag{3.5}
\]

\[
k_2 = h f(\psi_i + k_1/2, t_i + h/2)
\]

\[
k_3 = h f(\psi_i + k_2/2, t_i + h/2)
\]

\[
k_4 = h f(\psi_i + k_3, t_i + h)
\]

and \( h \) represents the time-step.

A history of RK methods by studying some of the early contributions was reviewed by Butcher (1996). In the present research work, RK4 was used to verify the solution of the ODEs obtained by applying the OCFE and ANN methods in order to ensure a robust numerical convergence.

### 3.4.2 Orthogonal Collocation on Finite Elements (OCFE)

Orthogonal collocation on finite elements (OCFE) was first proposed in 1971 by Paterson and Cresswell, and further developed in 1975 by Carey and Finlayson to solve the effectiveness factor problem for mass and heat diffusion with chemical
reaction in a catalyst pellet. Afterwards, a number of studies have looked at how OCFE could be used in a diverse range of applications (Gardini et al., 1985; Hairer and Wanner, 1999; Kiil et al., 1995; Lee et al., 1999). Details of the desirable properties of OCFE discretisation scheme in terms of accuracy and numerical stability is conducted in the work by Cuthrell and Biegler (1989).

In this work, OCFE was used as a solution scheme for the full discretisation of the dynamic models in a gene delivery optimal control problem. Following a direct-simultaneous approach and OCFE, both the state and control variable profiles were approximated through a set of polynomials on finite elements (Biegler, 1984; Neuman and Sen, 1973; Tsang et al., 1975). The dynamic optimisation problem was converted into a nonlinear programming problem (NLP) by discretising the state and control variables on finite times using Lagrange polynomials and Radau collocation points. As can be seen from Figure 3.1, the time domain is divided into a number of sub-domains, known as finite elements, and within each finite element, a number of specific time points, termed as collocation points, is considered. It has been demonstrated that Radau collocation points are commonly used for solving dynamic optimisation problems through direct collocation, since they allow constraints to be set at the end of each element and allow the system to be stabilised more efficiently if high level differential equations are present. The location of the collocation points can be provided by calculating the roots of the orthogonal polynomials (Biegler, 2007; Hairer and Wanner, 1999).

The numerical approximation of the state variables over the discretised time domain can be modelled by:
\[ \Psi_{k}^{OCFE}(t_{fe,cp}) = \Psi_{k}^{OCFE\text{in}}(t_{fe}) + h^{OCFE} \sum_{cp=1}^{n_{cp}} \Omega_{cp,cp} \frac{d\Psi_{k}^{OCFE}}{dt_{fe,cp}} \]  

where

\[ \Psi_{k}^{OCFE\text{in}}(t_{fe}) = \Psi_{k}^{OCFE\text{in}}(t_{fe-1}) + h^{OCFE} \sum_{cp=1}^{n_{cp}} \Omega_{cp,cp} \frac{d\Psi_{k}^{OCFE}}{dt_{fe-1,cp}} \]

and

\[ \frac{d\Psi_{k}^{OCFE}}{dt_{fe,cp}} = f_{k}(\Psi_{k}^{OCFE}, q^{OCFE}, t_{fe,cp}, \theta) \]

where \( \Psi_{k}^{OCFE}(t_{fe,cp}) \) and \( q_{k}^{OCFE}(t_{fe,cp}) \) are the \( k \)th state and control variables in element \( fe \in \{1, ..., N_{fe}\} \) at the collocation point \( cp \in \{1, ..., N_{cp}\} \), respectively. \( \Psi_{k}^{OCFE}(t_{fe}) \) is the initial value of the \( k \)th state variable at the beginning of finite element \( fe \). \( h^{OCFE} \) indicates the length of element \( fe \), \( \Omega_{cp,cp} \) is the collocation matrix, and \( \frac{d\Psi_{k}^{OCFE}}{dt_{fe,cp}} \) represents the value of the first derivative of the differential variable in every collocation point of each element \( (fe,cp) \).

### 3.4.3 Artificial Neural Network (ANN)

Artificial Neural Networks (ANNs) have been successfully applied in a wide range of chemical engineering applications, and provide a diverse range of solutions to fault detection problems, sensor data analysis, process system identification, and control (Himmelblau, 2008; Pirdashti et al., 2013). An artificial neural network is an information processing system which is made up of a number of highly interconnected network of artificial neurons (or nodes) and weighted connections. The ANN architecture is inspired by the structure of biological neural networks and has certain performance features in common with biological nervous systems (Mujtaba and Hussain, 2001; Yadav et al., 2015). In general, a network structure involves an input layer, one or more hidden layers, and an output layer. The basic structure of an ANN is shown in Figure 3.2. The model formulations provide a mapping sequence between inputs and outputs by obtaining the connection weights and biases of the neurons in the network. ANNs represent an attractive technology to deal with large datasets and approximate highly non-linear problems. They are empirical based modelling systems that can be tailored for multi-input–multi-output (MIMO) functions (Dua, 2010; Himmelblau, 2008; Hussain et al., 2003).
Chapter 3

A typical feed forward neural network aims to get started with an input layer that is connected to one or more hidden layers consisting of hidden neurons, and finally ends in an output layer (Prasad and Bequette, 2003).

![Artificial Neural Network (ANN)](image)

**Figure 3.2**: Artificial Neural Network (ANN).

Artificial neurons represent processing elements in the ANN framework. Each node takes one or more inputs over the connections and applies an activation function to its input to produce a relative output signal. The activation of a node is calculated by applying an activation function to the weighted sum of the input datasets plus a bias. Nodal weights and biases are the network parameters, which provide a bridge between different layers. An artificial neuron (hidden node) is represented in Figure 3.3 (Yadav et al., 2015).

The meshless ANN framework is capable to solve both ordinary and partial differential equations that relies on the approximation capabilities of feed forward neural networks. This computing system provides a solution written in a closed analytic form, which is differentiable and can be further used in subsequent calculations. This form of the solution hires a feed forward neural network as an approximation scheme, whose parameters are adjusted to minimise an error.
function, which can be solved by any optimisation techniques (Lagaris et al., 1998).

![Diagram of an ANN node/neuron](image)

**Figure 3.3:** Structure of an ANN node/neuron.

All inputs, \( x_i \), are multiplied to their assigned weights, \( \omega_{ij} \), and then summed up with a nodal threshold value known as the bias, \( b_j \), to form the net input to the artificial neuron, which is mathematically written as:

\[
net = \sum_{i=1}^{m} \omega_{ij} x_i + b_j
\]

The neuron acts as an activation function, \( f(net) \), that undergoes a nonlinear transformation using the neuron activation function or the transfer function to produce an output signal, \( N_k \), which can be expressed as:

\[
N_k = f(net) = f\left(\sum_{i=1}^{m} \omega_{ij} x_i + b_j\right)
\]

Different neuron activation functions, such as linear activation function, sigmoid activation function, sign function and step function, can be taken into consideration over the course of ANN model assembly (Yadav et al., 2015). A thorough discussion on description of the method will be presented in Chapter 6.

### 3.5 Parameter Estimation of Process Systems

With growing appreciation of modelling and optimisation in scientific and engineering
contexts, development of high-fidelity mathematical models has become more important to provide reliability, efficiency and safety in process systems. A critical part of the model construction, which leads to improvements in model-based process design, is to develop an efficient and reliable method for solving Parameter Estimation (PE) problems (Tjoa and Biegler, 1991). Several mathematical efforts have been done to understand in depth parameter estimation and the first step in this direction was made by Richard Bellman and collaborators in 1965 (Bellman et al., 1965).

The great majority of practical systems in biology, chemistry, physics, mathematics and control, have dynamic structures which can be modelled by differential equations (DE) involving unknown parameters that need to be estimated by using a set of measurements (experimental data). Parameter estimation of such models requires solving a dynamic optimisation (DO) problem (Biegler, 2007; Dua and Dua, 2012; Papamichail and Adjiman, 2004), which can be challenging, depending on the type of the model of the system and the choice of the objective function (Biegler et al., 1986; Englezos and Kalogerakis, 2001). Once the structure of a DE model is proposed, and a reliable solution technique is nominated for solving the DE systems (Biegler, 2007; Lagaris et al., 1998; Mazumder, 2015), an appropriate objective function must be chosen to determine the goodness of fit. Several forms of objective functions, ranging from a simple Least Squares (LS) function to complex nonlinear functions, can be selected for the PE problem (Bard, 1974). In the current work, we consider a PE problem formulated by taking the well-known estimation method of least squares in which the objective is to minimise the summed square of the residuals (the difference between the set of the measured data and the model predictions). The LS method has proven most practical criterion in estimation problems (Bard, 1974; Seinfeld and Chen, 1971). A typical structure of an estimation problem is given in Figure 3.4.

One important issue affecting the parameter estimation problem is parameter non-identifiability, meaning that a unique solution to the parameter estimation problem does not exist. Parameter non-identifiability is classified as structural and practical non-identifiability. The former is related to the model structure independent of experimental data while the latter takes into account the amount and quality of measured data (Raue et al., 2009). Several works in literature have considered the
identifiability issues. According to Degasperi et al. (2017), non-identifiability can be overcome by model reformulation or model reduction, or by generating additional data. Galvanin et al. (2013) proposed a general approach to investigate practical identifiability issues of PK/PD models, aiming for the optimal design of experiments in order to determine a proper set of experimental settings. There are also various methods available in literature to detect structural non-identifiability, which most of them are based on differential algebraic methods (Ljung and Glad, 1994).

Another challenge associated with solving the DO problem for estimating model parameters in differential equation systems is that the problem could be faced with a nonconvex feasible region. To avoid convergence of the program to a local optimum and exclude infeasible solutions, appropriate initial estimates should be provided by the modeller. In addition, tools of global optimisation are required to find a global optimum (Esposito and Floudas, 2000; Lin and Stadtherr, 2006, 2007; Nocedal and Wright, 1999; Singer et al., 2006).

Parameter estimation of ODE and PDE systems will be the focus of the next two sections.

3.5.1 Parameter Estimation of Ordinary Differential Equation Systems

Numerous relevant studies have been carried out to choose an appropriate scheme so as to yield consistently accurate parameter estimates for ODE systems. Several techniques, ranging from least squares methods (Bard, 1974), to multiple shooting
(Bock, 1981, 1983), collocation-based schemes (Biegler, 1984), approaches that apply spline functions (Michalik et al., 2009; Varah, 1982), and ANN-based methods (Dua, 2011; Dua and Dua, 2012), have been widely studied in this context.

Solving parameter estimation problems for ODE models is classified into decomposition algorithms and sequential/simultaneous algorithms. The decomposition approach (Varziri et al., 2008) is based on solving two optimisation problems in which the first one fits the measured data (experimental data) while the second one attempts to minimise the difference between the estimates of the derivatives achieved from the fitted model and the evaluated derivatives from the given model equations at experimental data points. Although decomposition approaches do not require discretisation of the ODEs while evaluating model parameters; however, a posteriori analysis should be performed by solving the ODEs for the obtained parameter estimates (Dua and Dua, 2012). Unlike the decomposition algorithm, sequential/simultaneous approaches require integration of differential equations. In a sequential method (Kim et al., 1991), the optimisation problem is separated from the ODE solution scheme while in a simultaneous algorithm, the dynamic system is transformed into a set of algebraic equations (AEs), which is a part of the overall optimisation problem resulting in a large-scale NLP (Dua and Dua, 2012; Tjoa and Biegler, 1991).

3.5.2 Parameter Estimation of Partial Differential Equation Systems

Since a wide range of real physical systems in many areas of applied science and industrial processes belong to Distributed Parameter Systems (DPS), their pertinent mathematical models often take the form of partial differential equations (PDEs) describing the spatial-temporal dynamics of the system. A survey of literature revealed that while the forward problem of approximating solutions to PDE systems for given values of parameters had been extensively studied, a relatively sparse literature had been devoted to the inverse problem of estimating model parameters based upon the experimental data (observed state variables) to explore the potentiality of ANN-based formulations which consequently forms the main objective of the last part of the thesis. Developing a reliable parameter estimation method for PDE systems is crucial to obtain accurate parameter values with fast convergence rates to advance a proper mathematical model of the process such that the model
Chapter 3

predictions could confirm the underlying dynamic behaviour of the process.

Challenges associated with parameter estimation of DPS arise when an attempt is made for the system modelling. DPS modelling is classified into two cases: parameter estimation for known structures and parameter estimation for unknown structures. For the case in which the PDE model structure of the system is known, which is the main purpose of this work, a traditional parameter estimation problem is required to be solved in which the least squares method or any other optimisation methods can be used to estimate the unknown system parameters (Coca and Billings, 2000; Mohan and Datta, 1991; Muller and Timmer, 2004). On the other hand, as for unknown processes where the model structure is not specified, there is no standard solution, therefore more efforts are required because of the complexity and large varieties. Note that solving parameter estimation problems for unknown PDE model structures is beyond the scope of this work and thus the interested reader is referred to a work by Li and Qi (2010) reviewing different solution options for such models.

Similar to traditional PE problems, selecting the most suitable numerical approximation approach to solve the partial differential equations and choosing a proper criterion for the determination of unknown model parameters will be the key challenges for this part of the work. A few of the previous works in this context will be reviewed here.

Beck (1970 a, b) employed the popular finite difference method (FDM) to provide an approximate solution to PDEs and then applied the least squares method to estimate the physical properties in the heat conduction equation. The work carried out by Seinfeld and Chen (1971) had looked at the parameter estimation techniques based on the method of steepest descent, quasilinearization, and collocation in the class of PDE problems of chemical engineering interest. Polis et al. (1973) presented a methodology in which Galerkin’s method had been used to convert the PDEs into a set of ODEs. The authors applied three optimisation schemes including a steepest descent method, a search technique and nonlinear filtering, for estimating the unknown parameters. The purpose of this was to show that the PDE parameter estimation problems could be transformed into a standard optimisation problem in which any optimisation algorithms can be applied. More early reviews are given by Polis and Goodson (1976) and Kubrusly (1977). In the survey by Kubrusly (1977),
Identification methods for the DPS are classified into three classes: (i) direct method, (ii) reduction to Lumped Parameter Systems (LPS), and (iii) reduction to Algebraic Equations (AE). The direct method utilizes the infinite-dimensional system model to obtain the parameters. The reduction-based methods, which is also known as time-space separation, involve spatial discretisation in order to reduce the PDEs into a set of ODEs in time to which estimation methods for LPS can be applied (Hidayat et al., 2017). A number of other relative works exist in literature including statistical methods (Banks and Kunisch, 1989; Fitzpatrick, 1991; Xun et al., 2013), Laguerre-polynomial approach (Ranganathan et al., 1984), general orthogonal polynomials (Lee and Chang, 1986), Fourier series method (Mohan and Datta, 1989), singular value decomposition (Gay and Ray, 1995), artificial neural networks coupled with traditional numerical discretisation techniques (Gonzalez-Garcia et al., 1998), and extended multiple shooting method (eMSM) (Muller and Timmer, 2002).

While previous contributions on the inverse problem of estimating unknown parameters in pre-selected PDE systems have investigated extensively the PE properties such as accuracy and computing time; they discuss cases where methods mainly consider functions over a uniform grid discretisation; so, PDE models with irregular boundaries were largely ignored. Further advances in terms of estimation accuracy and savings in computation time are the other potential areas of improvements in this context. For this purpose, more attention needs to be paid to the numerical solution strategies of partial differential equations.

Several methods can be used for solving a system of partial differential equations. In general, a very limited number of PDE problems can be solved analytically due to the inherent complexity of distributed systems. Therefore, mathematicians have explored and developed a number of numerical approximations for the solution of PDE systems such as the method of weighted residuals (Finlayson and Scriven, 1966), finite difference methods (Mazumder, 2015; Smith, 1985), the numerical Method of Lines (MOL) (Schiesser, 1991), finite element methods (Bathe, 1996), Finite Volume Methods (FVM) (Mazumder, 2015), and artificial neural networks (Lagaris et al., 1998). Xu and Dubljevic (2017) recently developed a methodology based on the Model Predictive Control (MPC) algorithms for linear transport-reaction models. The authors proposed Cayley-Tustin transformation as an exact time discretisation scheme, and then developed a model predictive control formulation to account for
the spatial nature of the problem.

Among the available solution strategies for simulation of PDE models, in this work, an artificial neural network (ANN) architecture was used to solve the partial differential equations because of its excellent performance (Lagaris et al., 1998). ANN-based formulations represent an exciting avenue of research as they offer meshless frameworks to account for irregular boundaries. An ANN model involves parameters such as weight matrices and bias vectors that are adjusted to minimise a suitable error function. The computation of the network parameters in the ANN model forms part of the solution of the PDEs. So, the original parameter estimation problem for PDE systems becomes an optimisation problem in which the objective is to simultaneously approximate the PDE models by computing the ANN network parameters, and estimate the PDE model parameters such that the model predictions being in a good agreement with the measured data (experimental observations). Comprehensive experience in ODE parameter estimation (Dua, 2011; Dua and Dua, 2012) indicates that ANN-based methodology was effectively and successfully tested for ODE systems, and thus is a candidate for parameter estimation of PDEs. In this work, the parameter estimation problem is studied in terms of a class of linear and nonlinear elliptic and parabolic PDE systems with different boundary conditions (Dirichlet, Neumann and Robin). In Chapter 6, a general formulation of the proposed method will be described and numerical examples will be presented to validate the applicability of the methodology.

3.6 Summary

This chapter has detailed an overview of differential equations, optimal control theory and solution strategies for dynamic optimisation problems, numerical solution methods for differential equations, and parameter estimation. The development of high-fidelity mathematical models has a key role in advanced design and control of biological and chemical process engineering systems. Therefore, the rest of this thesis builds on the provided theoretical basis in this chapter, in order to establish solutions for the simulation of dynamic models, parameter estimation problems, and the gene delivery optimal control problem.

A review of the literature for parameter estimation of partial differential equation systems showed that no concerted effort has been made to investigate the
potentiality of ANN frameworks on this topic. Therefore, Chapter 6 will extend the state of the art on the inverse problem of estimating model parameters in PDE systems by addressing the shortcomings highlighted in Section 3.5.2. A simultaneous solution and optimisation strategy will be presented via ANNs to account for non-uniform arbitrary regions which widely exist in real-world phenomena such as complex cellular architectures (Dinh et al., 2007; Dreij et al., 2011). According to Dinh et al. (2007), much of the complexity involved in a vector-cell system is associated with the spatiotemporal distribution of vectors in an intracellular environment. Therefore, a spatial view of the cell is required to be considered, and so spatial coordinates can be introduced to represent the cell geometry, which will be handled using partial differential equations (PDEs). Cell topology strongly influences the spatiotemporal distribution of gene carriers, and thus, their optimal intracellular pathway. This work will provide a stepping stone for further investigation of the effect of cellular geometry in the diffusion of genetic materials by considering PDE models with irregular boundaries to take into account complex cellular architectures. To this end, the spatiotemporal image correlation spectroscopy can offer an overall picture of the dynamic and spatial nature of intracellular trafficking of carriers (Kulkarni et al., 2005).
4 OPTIMAL MODEL-BASED CONTROL OF NON-VIRAL siRNA DELIVERY

4.1 Introduction

Optimisation of siRNA design algorithms along with combinations of chemical modifications can reduce the immunogenicity or off-target effects while improving the stability of siRNAs to silence target genes (Kanasty et al., 2012). Despite considerable progress in the optimisation of siRNA delivery protocols, safe and efficient delivery of siRNAs is still a key challenge, which has limited the scope of successful application of siRNA therapy in humans (Semple et al., 2010).

This chapter extends the state of the art on model-based control of gene transfer systems by addressing the short-comings highlighted in Section 2.5.3 in Chapter 2.
An integrated PK/PD modelling platform for both efficacy and safety is developed based on *in vitro* experimental analysis to provide quantitative understanding of non-viral siRNA delivery. The developed PK/PD models are then used for an optimal control formulation that is set up to optimise siRNA delivery for *in vivo* conditions. The proposed modelling and control approach effectively computes an optimal infusion rate of siRNA therapeutics while simultaneously considering the key pharmacological issues. Unlike many previous studies that partially describe the complexity of gene delivery processes (Banks et al., 2003; Ledley and Ledley, 1994; Varga et al., 2001, 2005), the developed mathematical modelling and control framework in this research study provides an effective trade-off decision-making platform for siRNA delivery to take into account the main multi-objective optimisation issues, such as efficacy, toxicity, and the influence of uncertainty in cell division time. Effect of variations in cell doubling time is explored by incorporating time constraints into the optimisation problem to achieve maximum desired effects before cell division occurs. A number of dynamic simulations are also performed comparing continuous infusions with bolus injections of siRNA therapeutics. According to the obtained results, a single bolus administration of therapeutic agents is not optimal for obtaining a persistent reduction in expression of defective genes. Therefore, maximum therapeutic effect with minimal toxicity is manifested with an optimal continuous infusion of siRNAs over time, before cell division takes place.

### 4.2 Methods

#### 4.2.1 Mathematical Modelling of siRNA Delivery

A pharmacokinetic model was developed based upon the available experimental data to represent intracellular transport processes responsible for delivery of siRNA during *in vitro* cell transfection. The PK model was then modified to include the infusion rate of siRNAs for *in vivo* delivery. A pharmacodynamic model was constructed consistent with published data to study the relationship between siRNA concentration and pharmacological effects. The developed PK model, coupled with the PD model, provided an integrated PK/PD modelling platform that was used for a multi-objective optimisation framework in the presence of different practical constraints in order to obtain an optimal siRNA delivery infusion profile for *in vivo* conditions.

Development of high-fidelity mathematical models involves parameter estimation in
which the objective is to minimise the summed square of the difference between the set of experimental data and the model predictions (Englezos and Kalogerakis, 2001). The focus of this chapter is on a gene delivery system that follows pharmacokinetic modelling approach involving ordinary differential equations (ODEs). Parameter estimation of such systems requires solving a dynamic optimisation problem. In this work, a simultaneous parameter estimation approach was performed using ANN approximations (Dua and Dua, 2012). Orthogonal Collocation on Finite Elements (OCFE) was then used as a solution scheme for the full discretisation of the dynamic models in the gene delivery optimal control problem. For the purpose of comparison, the dynamic simulations were validated using a well-known fourth order Runge–Kutta (RK4) scheme and the meshless ANN framework. All the optimisation problems were formulated as nonlinear programming (NLP) problems and solved using the General Algebraic Modeling System (GAMS), a modelling system for mathematical programming and optimisation (Brooke et al., 1998).

4.2.2 Experimental Data

In an experimental study by Zhou et al. (2013), SK Hep-1 cells (human hepatocellular cell line) were transfected with siRNA/NC complexes, and the overall cellular and cytoplasm exposure of siRNA were reported over a 24-hr period as a function of time. The authors analysed the cellular pharmacokinetics of siRNA for a novel type of nanocarrier, SPANosomes (SP) formulation. They also reported the gene silencing activity and cytotoxicity of siRNA/NC complexes. In the current computational work, the published experimental data was used to develop an integrated PK/PD model that describes the kinetic pathways of nanocarrier-mediated disposition of siRNA so as to effectively predict the siRNA exposure to its site of action while improving pharmacological effects.

4.2.3 Pharmacokinetic Modelling

Mathematical representations of the PK model were used for quantitative evaluation of in vitro transfection. There are several biological barriers for NC-mediated siRNA delivery to reach their intended targets. The focus of this work is on intracellular barriers where the developed PK compartmental model, which is based upon the available experimental data, includes two compartments: the endosome and the cell cytoplasm. Figure 4.1 shows the model structure in which siRNA therapeutics can be
transferred in one direction across the barriers.

\[ \frac{d}{dt} E(t) = -\theta_1 E(t) \]  
\[ \frac{d}{dt} C(t) = \theta_1 E(t) - \theta_2 C(t) \]

where \( E(nM) \) and \( C(nM) \) are state variables and represent the siRNA concentration in the endosome and in the cytoplasm, respectively. \( \theta_1(hr^{-1}) \) and \( \theta_2(hr^{-1}) \) are rate constants controlling the transport of siRNA from the endosome to the cytoplasm \((\theta_1)\) and trafficking through the cytoplasm in order to be loaded onto RNAi machinery \((\theta_2)\).

4.2.4 Pharmacodynamic Modelling and Optimal Control of siRNA Delivery

4.2.4.1 Gene Silencing Activity

In *in vivo* conditions, siRNA/NCs can be infused over a period of time (Figure 4.2). So, Equation 4.1 is modified as follows:
\[
\frac{d}{dt} E(t) = -\theta_1 E(t) + q(t)
\]

where \(q(t)\) (nM/hr) is the flow rate of the siRNA therapeutics infused.

\[I(\%) = (I_{\text{max}} + I_0) - I_{\text{max}} \times \frac{C_{\text{siRNA}}}{C_{\text{siRNA}} + IC_{50}}\]

where \(I\) is the inhibitory effect, \(I_{\text{max}}(\%)\) represents the maximum inhibitory effect, \(I_0(\%)\) is a baseline effect parameter when maximum therapeutics are present in the cell, and \(IC_{50}\) (nM) is the siRNA concentration required to produce 50\% of the maximum inhibitory effect. To estimate the pharmacodynamic parameters, the inhibitory effect model, Equation 4.4, was fitted to the relative gene expression values, which had been experimentally observed in vitro by Zhou et al. (2013).

**4.2.4.2 Cytotoxicity**

Cellular toxicity is investigated by considering a population of alive cells known as Cell Viability, which is controlled by two processes: production of cells and cell loss.

Cell viability = production of cells – cell loss

To describe the cytotoxic effects, the sigmoid Hill equation model has been modified
Chapter 4

to a composite $E_{\text{max}}$ model to include a no-drug response ($CV_0$) and concentration effects over time. The following relationship was constructed such that the both processes of cell production and cell loss are functions of total siRNA concentration in the cell:

$$CV(\%) = CV_0 + \frac{G_{\text{max}} \times C_{\text{siRNA}}^\gamma}{GC_{50}^\gamma + C_{\text{siRNA}}^\gamma} - \frac{T_{\text{max}} \times C_{\text{siRNA}}^\gamma}{TC_{50}^\gamma + C_{\text{siRNA}}^\gamma}$$ 4.6

where $CV$ denotes the cell viability indicating the percentage of alive cells during the therapy, $CV_0(\%)$ represents the initial percentage of alive cells, $G_{\text{max}}(\%)$ is the maximum percent growth, $GC_{50}(nM)$ is the siRNA concentration required to produce 50% of the maximum percent growth, $T_{\text{max}}(\%)$ denotes the maximum toxicity, $TC_{50}(nM)$ is the siRNA concentration required to produce 50% of the maximum toxicity, and $\gamma$ is the power parameter to account for the curvature. The situation of combined drug action occurs when a single drug performs simultaneously at two different receptors (Gabrielsson and Weiner, 2010). The CV model, Equation 4.6, represents a biphasic concentration-effect relationship involving two phases: phase A corresponded to the protective effect on cell viability (production process), whereas phase B reflected the cytotoxicity (loss process). The former phase is represented by the $G_{\text{max}}$ term and the latter phase is represented by the $T_{\text{max}}$ term. In this work, cell viability values, which had been experimentally observed by Zhou et al. (2013), were fitted to the developed CV model in order to obtain the corresponding pharmacodynamic parameters.

4.2.4.3 Optimal Control

The siRNA delivery optimal control problem is formulated and solved subject to the system models and a set of constraints for computing an optimal infusion rate at optimal times. To this purpose, a multi-objective optimisation framework is applied dealing with a number of objective functions to be optimised simultaneously. The siRNA delivery optimal control problem is of the following form:

$$\min_{q(t)} \text{TIE} = \int_{t=0}^{t=t_f} I(t) \, dt \equiv \Delta t \sum_{t=0}^{t=t_f} I(t)$$ 4.7

$$\max_{q(t)} CV(t)$$ 4.8

subject to the system models and initial conditions; where TIE represents the total
inhibitory effect, and \( t_f (\text{hr}) \) is the final time at the end of the therapy. The first objective, minimisation of TIE, aims to reduce undesirable gene expression, while the second objective, maximisation of \( CV(t) \), aims at minimising cytotoxicity by preserving the life of the cells. The above multi-objective optimal control problem is reformulated as an \( \varepsilon \)-constrained optimisation problem (Clark and Westerberg, 1983), with the total inhibitory effect treated as the primary objective to be minimised, and the cell viability treated as a constraint with the limits varied between 50-100%, which can be modelled as follows:

\[
\min_{q(t)} \int_{t_0}^{t_f} I(t) \, dt \cong \Delta t \sum_{t=0}^{t_f} I(t) \quad 4.9
\]

subject to: \( CV(t) \geq CV^{LO} \), Equations 4.2-4.4, 4.6, and initial conditions.

where \( CV^{LO}(\%) \) is the minimum acceptable cell viability level. A number of case studies are presented in section 4.3 to demonstrate the advantages of the proposed model-based optimal control framework for siRNA delivery.

A conceptual block diagram representing a closed-loop model-based control scheme is shown in Figure 4.3. Depending on the disease type, the patient output variable of interest could be measured and supplied to a control algorithm. The algorithm would calculate an optimal infusion rate to keep the pharmacological effects at desirable levels, and a signal would drive an infusion pump to deliver the exact optimal amount of therapeutic materials. Disturbance can be attributed to surgical or other stimuli including the lifestyle of a patient.

![Figure 4.3: Block diagram for a model-based optimal control of siRNA delivery.](image)

### 4.3 Results

Consider the following PD parameters, which were reported by Zhou et al. (2013),
$I_0 = 5.2\%$ and $IC_{50} = 5.5\text{ nM}$. $I_{\text{max}}$ was obtained as 94.8% by fitting the relative gene expression values to the inhibitory effect model given by Equation 4.4. Cell viability values were also fitted to the CV model (Equation 4.6) to obtain the following PD parameters: $G_{\text{max}} = 21.2\%$, $GC_{50} = 15.4\text{ nM}$, $T_{\text{max}} = 71.7\%$, $TC_{50} = 94.9\text{ nM}$, and $\gamma = 7$. The developed PK/PD models in this work were validated by comparing their predictions to experimental measurements, which are demonstrated in Figure 4.4 and Figure 4.5. The schematic illustration of the composite $G_{\text{max}} / T_{\text{max}}$ model is also shown in Figure 4.6, indicating that the estimated PD parameters in this study are in accordance with the system biology. Sensitivity analysis have also been performed (Figure 4.7) to determine the relative impact of each parameter on the CV model output. The role of each parameter in the model was determined by varying only one parameter at a time while keeping all the other parameters constant, set at their estimated values. From the analysis, the maximum percent growth parameter, $G_{\text{max}}$, turned out to be the most sensitive parameter.

![Figure 4.4: Comparison of the pharmacokinetic experimental results (represented the mean ± S.D.) and the PK model predictions.](image-url)
Chapter 4

Figure 4.5: Comparison of the cell viability experimental results (represented the mean ± S.D.) and the model predictions.

![Comparison of cell viability results](image)

**Figure 4.5**: Comparison of the cell viability experimental results (represented the mean ± S.D.) and the model predictions.

**Figure 4.6**: Schematic illustration of the composite $G_{\text{max}}/T_{\text{max}}$ model. The $G_{\text{max}}$ and $T_{\text{max}}$ values were estimated to be 21.2% and 71.7%, respectively; $G_{50}$ and $T_{50}$ are 15.4 nM and 94.9 nM, respectively, and the exponent is 7.

As can be seen in Figure 4.7, the CV model was simulated by (a) varying $G_{\text{max}}$ and keeping the other parameters constant at their estimated values - $G_{\text{max}}$ was varied ~5% staring from 5% (baseline value) and going up to 35%; (b) varying $G_{50}$ and keeping the other parameters constant at their estimated values - $G_{50}$ was varied ~4 nM staring from 3 nM (baseline value) and going up to 27 nM; (c) varying $T_{\text{max}}$ and keeping the other parameters constant at their estimated values - $T_{\text{max}}$ was varied ~10% staring from 40% (baseline value) and going up to 100%; (d) varying $T_{50}$ and keeping the other parameters constant at their estimated values - $T_{50}$ was varied ~
\(\sim 5nM\) starring from \(70nM\) (baseline value) and going up to \(99nM\); and (e) varying \(\gamma\) and keeping the other parameters constant at their estimated values. - \(\gamma\) was varied \(\sim 1\) staring from 1 (baseline value) and going up to 7.

Figure 4.7: Sensitivity analysis of the model parameters.
Chapter 4

As cells were transfected for 4 hr followed by 44 hr incubation, a 48 hr time frame \((t_f = 48 \text{ hr})\) was assumed for the simulation and optimisation problems. Intracellular exposure of siRNA, gene silencing activity, and cytotoxicity resulting from two different delivery modes are compared and reported in sections 4.3.1 and 4.3.2. PK/PD profiles are observed following a bolus administration of siRNA therapeutics and a continuous infusion over a period of time.

### 4.3.1 Bolus Injection

Bolus administration is a rapid injection when all of the dose is given in a short period of time. In this section, single-dose bolus injections were simulated and the model was implemented in GAMS. Figure 4.8 shows the siRNA concentration in the cell, inhibitory effect, and cell viability as a function of time, after start of treatment with total injected doses of 80, 100, 150, and 250 nM.

![Graphs showing PK/PD responses to bolus injections of siRNA](image)

**Figure 4.8**: Pharmacokinetic and pharmacodynamic responses to bolus injections of siRNA therapeutics after start of treatment with total injected doses of 80, 100, 150, and 250 nM. (a) Time profile of siRNA concentration in the endosome. (b) Time profile of siRNA concentration in the cytoplasm. (c) Time profile of inhibitory effect. (d) Time profile of cell viability.

Time profiles of siRNA concentration in the endosome and cytoplasm are shown in Figure 4.8a and Figure 4.8b, respectively, when the therapeutic is administered as a bolus dose. The observed reduction of siRNA concentration over time for all four
different doses in the endosome is due to the cellular distribution and irreversible elimination of the compound. siRNA therapeutics must be capable of escaping the endosome–lysosome degradation axis and releasing from their carriers to the cytoplasm so as to be loaded onto RNA-induced silencing complex (RISC) (Wang et al., 2010).

Pharmacological responses depend on the total siRNA concentration in the cell, so minimum inhibitory effect can be achieved once a bolus dose of therapeutics is administered (Figure 4.8c). The decrease in the total inhibitory effect appeared to be dose-dependent. Increasing the infusion from a low dose of siRNA therapeutics (80 nM) to intermediate doses (100 and 150 nM), and finally to a high dose (250 nM) will decrease the total inhibitory effect (Figure 4.8c). For more details see Figure 4.9.

According to Figure 4.8d, the reduction in cell viability is associated with high-dose bolus injections. No significant cytotoxicity was observed in the low injected dose (80 nM); however, higher dose of siRNA therapeutics could decrease cell viability in a dose-dependent manner.

There is a rapid rise in the inhibitory effect profile while siRNA concentration is getting decreased over time (Figure 4.8c). Therefore, a single bolus infusion of siRNAs resulted in a transient dose-dependent decrease in inhibitory effect that influences on the total inhibitory effect to become about 10 times greater for bolus
injection, suggesting an optimal continuous siRNA infusion is more favourable delivery mode. The observed PK/PD profiles following a continuous infusion over a time frame of 48 hr are reported and discussed in the next section.

Note that, the OCFE-based simulation results were validated by comparisons with ANN and RK4 simulation values and the results are shown in Figure 4.10.

Figure 4.10: Comparison of the GAMS OCFE/ANN/RK4 simulation results for siRNA delivery over 48-hour transfection.

4.3.2 Optimal Control of siRNA Delivery

The siRNA delivery optimal control problem (Equation 4.9) was solved for lower bound values of 50, 60, 70, 80, 90, and 100 % that were placed on cell viability. Figure 4.11 shows the multi-objective optimisation results describing the trade-off between the minimum total inhibitory effect and lower bounds on cell viability. A relaxation on $CVLO$ results in a decrease in the minimum total inhibitory effect that can be achieved (Figure 4.12c), and an increase in the infusion rate (Figure 4.12a). Typically, there is a conflict between efficacy and toxicity. Minimal inhibitory effect cannot be achieved without sacrificing the safety.
According to the obtained results and comparing the six different case studies of various lower bounds, increasing the siRNA infusion rate would lead to a reduction in the inhibitory effect while increasing the risk of toxicity induced cell death (Figure 4.12). An optimal infusion rate was computed such that the total inhibitory effect was minimised and bounds on cell viability were respected. Figure 4.12a shows the optimal siRNA concentration that can be infused over a 48 hr period of time to achieve maximum gene silencing activity (Figure 4.12c) while maintaining cell viability at desirable levels (Figure 4.12d).

The control optimisation problem was solved subject to the constraints on the cell viability. Adding sensible bounds is necessary to ensure that minimum toxicity can be achieved, while still reaching maximum knockdown efficacy (Figure 4.12d). According to Zhou et al. (2013), the production process is presumed to occur because low concentrations of siRNA/NC complexes can stimulate cell metabolic activity that leads to increase the apparent cell viability in comparison with the untreated group. However, the loss process could be due to the fact that the cells may die if the level of toxicity rises above a certain level. Undesirable effects such as toxicity result from the interactions between biological components and foreign materials such as siRNA molecules, gene carriers alone or in formulation with siRNAs (Kanasty et al., 2012; Lv et al., 2006; Nel et al., 2009). Here, the observed cytotoxicity is probably due to the surfactant activities of Span 80 or the other compositions of the SP formulation. Zhou et al. (2013) reported that the helper component in the SP formulation might make an important contribution to the
cytotoxicity of the NCs. However, siRNA molecules can also elicit adverse biological effects including immune stimulation resulting in inflammatory responses and off-target silencing leading to toxicity (Kanasty et al., 2012; Wang et al., 2010; Xue et al., 2014). Figure 4.12b shows the exposure of siRNAs in the cytoplasm. siRNA therapeutics are first released in the endosome and then dispersed throughout the cytoplasm. As time passes, siRNA concentration in the endosome is shown to rise at a rapid pace suggesting that the infused siRNAs accumulate in the endosome upon their arrival. After infusing the genetic materials, a fraction of siRNAs is escaped from the endosome into the cytoplasm and gets distributed throughout the cytoplasm and finally transferred to the site of action in order to elicit their biological effects.

![Figure 4.12](image)

**Figure 4.12:** siRNA delivery optimal control results and the pharmacodynamic responses to siRNA infusion over 48 hr transfection for different lower bounds on cell viability when practical limitations of gene delivery devices are not imposed. (a) Time profile of optimal siRNA infusion. (b) Time profile of siRNA concentration in the cytoplasm. (c) Time profile of inhibitory effect. (d) Time profile of cell viability.

Depending on the practical limitations of gene delivery devices, different constraints can be introduced into the optimisation model. Two such examples are (i) constraints on the process control variable, for example, the maximum value that infusion can take, and (ii) the incremental change in infusion rate, $\Delta q(t)$, can be constrained between certain lower and upper bounds. In this section, a case study was first
considered when \( q \) was unconstrained to investigate the controlled siRNA delivery when practical limitations were not imposed. The siRNA delivery optimal control problem was also formulated and solved where practical limitations of gene therapy devices were imposed thereby an important constraint was introduced and incorporated into the control framework:

\[
0 \leq q(t) \leq q^{\text{max}}
\]

where \( q^{\text{max}} \) represents an upper bound on the infusion rate indicating the maximum value that infusion can take during the therapy. In the presence of constraints on \( q \), that is, \( q^{\text{max}} = 30 \text{ nM/hr} \) or \( q^{\text{max}} = 40 \text{ nM/hr} \), while lower bound of 100% on cell viability is respected, the results are shown in Figure 4.13. For both cases, a maximum allowable value of infusion takes place initially, which drops with time and then increases to a plateau of 21.85 nM/hr for the rest of the therapy to achieve persistent gene silencing with minimum adverse side effects (Figure 4.13).

The observed initial spike in the siRNA infusion rate in Figure 4.12a, which decreases rapidly during the first hour of infusion, revealed the need to introduce another important constraint into the formulation. So, incremental change in infusion
rate, $\Delta q(t)$, was constrained between certain lower and upper bounds of 1 nM/hr per collocation point for three collocation points in a finite element using OCFE, which is modelled as follows and the results are shown in Figure 4.14.

$$\Delta q_{\text{min}} \leq \Delta q(t) \leq \Delta q_{\text{max}}$$

This constraint was proposed to reduce problems associated with practical limitations of infusion devices in which the transfer of a large amount of therapeutics over a short period of time would be impossible. The observed results suggest that the system could be controlled in the presence of different constraints. When practical limitations are imposed, a high value of siRNA infusion takes place initially, which eventually decreases to a plateau, to obtain minimum total inhibitory effect over the therapy (Figure 4.14c).

![Figure 4.14](image_url)

**Figure 4.14**: siRNA delivery optimal control results and the pharmacodynamic responses to siRNA infusion over 48 hr transfection when practical limitations of infusion devices are imposed. (a) Time profile of optimal siRNA infusion. (b) Time profile of siRNA concentration in the cytoplasm. (c) Time profile of inhibitory effect. (d) Time profile of cell viability.

### 4.3.3 Incorporating Time Constraints

The proposed model-based optimal control framework in the previous section addressed siRNA delivery to non-dividing cells. The aim of this section is to address the issues pertaining to the presence of constraints in the developed models to study the effects of cell division and uncertainties in cell doubling time. A generic
formulation for non-dividing conditions was presented in section 4.2.4 where constraints on process variables were incorporated into the siRNA delivery optimal control problem. Another critical constraint is on the time to take into account the cell multiplication so that the therapeutic effect is manifested before cell division takes place. To this purpose, the developed PK/PD models and control framework were used to incorporate the required constraints in order to deal with disruptions of cell proliferation. If the siRNAs inside the cytoplasm are loaded onto RNAi machinery and exert their therapeutic effects before the time of cell division, the desired effects will remain in the newly formed cells. So, the length of the whole cell cycle should be considered in the formulation, as the site of action for siRNA therapeutics is the cytoplasm. According to research findings from literature (Ling et al., 2012), the doubling time for SK HEP-1 cells is approximately 25 hr, so optimal concentration of siRNA therapeutics must be delivered to the cytoplasm before cell division takes place. It is then possible to set an optimal control problem, by defining time constraints. According to Sandler et al. (2015), there is variability in cell cycle duration as different conditions could affect various stages of the cell cycle. For instance, in this study, low concentrations of siRNA/NC complexes can stimulate cell metabolic activity (Zhou et al., 2013) that could affect cell cycle in SK HEP-1 cells in comparison with the untreated group. To assess if uncertainties in doubling time affected the siRNA delivery process, a constrained optimisation problem was developed and solved while considering different cell doubling time ($T_d$) values of 4, 10, 15, 20, and 25 hr. Cell division greatly affects transfection (Martin et al., 2014), so the duration of therapy relies on the cell doubling time. Infusion must be completed before cell division takes place in order to achieve more efficient therapy. Therefore, siRNA therapeutics are assumed to be infused over the period of time required to obtain maximum desired effects, so the infusion is stopped at the doubling time while the algorithm considers the system performance for further 2 hr. These assumptions were made for all case studies in this section and they can potentially be modified under different experimental conditions.

Figure 4.15a shows the time profile of optimal siRNA infusion for different cell doubling times. As the doubling time increases, the amount of infusion increases. Longer transfection times require more siRNAs to keep the therapeutic effect at a desirable level. As it is signified in Figure 4.15a, a large amount of siRNAs can be infused at the start of treatment to get maximal knockdown while minimising
cytotoxicity. Minimum inhibitory effect is reached before cell division takes place. Once cells divide, siRNA infusion is discontinued and remains steady at 0 nM/hr toward the end of the study resulting in increase in both inhibitory effect and cell viability (Figure 4.15).

Figure 4.15: Optimal control results and the pharmacodynamic responses to siRNA infusion over different transfection time periods to study the effect of uncertainty in cell division time when practical limitations are not imposed and $q$ is unconstrained. (a) Time profiles of optimal siRNA infusion. (b) siRNA concentration-time profiles in the cytoplasm. (c) Time profiles of inhibitory effect. (d) Time profiles of cell viability.

According to Zhou et al. (2013), only the portion of siRNA therapeutics that is released from the carriers into the cytoplasm is considered as determinants for gene silencing activity. The results in Figure 4.15b present the optimal siRNA exposure at the target compartment, cytoplasm, for exerting maximum therapeutic efficacy over the therapy. Constraints on the CV model were proposed to address issues associated with cytotoxicity. The cell viability is increased rapidly just after the first injection suggesting that low concentrations of siRNA/NC complexes stimulate cell metabolic activity leading to an increase in the apparent cell viability (Zhou et al., 2013). However, as the siRNA concentration in the cell (Figure 4.15b) increases, cell viability decreases and remains bounded until cell division occurs (Figure 4.15d).
From the observed results, it can be concluded that the model-based optimal control methodology provided a balance between efficacy and toxicity for siRNA delivery, while considering the effect of uncertainties in cell division time on intracellular transport.

The current findings have provided valuable information; however, challenges remain such as capability of infusion devices to deliver a large amount of therapeutics for different time frames. So, the developed model was reformulated as the control variable was constrained to investigate the behaviour of the system over different time frames while simultaneously considering efficacy, toxicity, cell division, and the uncertainty in cell division time, which allows for representing siRNA delivery with wider scope. The optimal control results and the pharmacodynamic responses of this case study are presented in Figure 4.16 in which the maximum value that infusion can take is 40 nM/hr.

**Figure 4.16:** Optimal control results and the pharmacodynamic responses to siRNA infusion over different transfection time periods to study the effect of uncertainty in cell division time when practical limitations are imposed and q is constrained. (a) Time profiles of optimal siRNA infusion. (b) siRNA concentration-time profiles in the cytoplasm. (c) Time profiles of inhibitory effect. (d) Time profiles of cell viability.

In this case, the total inhibitory effect is higher than that of achieved for
unconstrained \( q \). Once the therapy is initiated, \( q \) takes the maximum allowable value of 40 nM/hr, which then drops over the first hours of therapy. The siRNA infusion rate depends upon the transfection time periods. As the cell doubling time increases, longer transfection times are required, so more siRNA therapeutics must be infused to keep the inhibitory effect and the cell viability at desirable levels (Figure 4.16c and Figure 4.16d). The work has also considered another scenario in which the maximum value that infusion can take is 30 nM/hr, and the corresponding results are shown in Figure 4.17.

![Figure 4.17: Optimal control results and the pharmacodynamic responses to siRNA infusion over different transfection time periods to study the effect of uncertainty in cell doubling time when practical limitations are imposed and \( q \) is constrained. (a) Time profiles of optimal siRNA infusion. (b) siRNA concentration-time profiles in the cytoplasm. (c) Time profiles of inhibitory effect. (d) Time profiles of cell viability.](image)

The novel application of the optimal control strategy in siRNA delivery systems aims to simultaneously describe the intracellular transfection process and incorporate the main multi-objective optimisation issues such as efficacy and toxicity, as well as uncertainties in cell doubling time. The controller is robust to uncertainties in cell division time, however requires retuning for a case of large patient variations. The developed integrated modelling platform can be simply adapted for a wide range of conditions such as different carriers and various practical limitations.
4.4 Conclusions

An optimal delivery of siRNA-based therapeutics into the site of action is critical for the safety and efficacy of RNAi therapy for patients suffering from diseases that are associated with undesirable gene expression. This computational study begins with the development of an integrated PK/PD model, consistent with the level of available experimental data, demonstrating the time–concentration–effect relationship for siRNA/NC complexes. The siRNA delivery by non-viral nanocarriers was modelled as biochemical reactions illustrating the critical steps involved in the delivery. This is favourable as it allows us to study the mechanisms of siRNA delivery by determining the rate-limiting steps in NC-mediated delivery. From a kinetic point of view, the rate constants obtained from the quantitative analysis of in vitro experimental data (Table 4.1), suggest that the possible rate-limiting step in siRNA delivery is the endosomal escape, which is consistent with the work by Gilleron et al. (2013).

Table 4.1: A summary of the parameter estimation problem.

<table>
<thead>
<tr>
<th>PK model equations</th>
<th>Estimated θ values (h⁻¹)</th>
<th>LS objective function</th>
<th>CPU time (s)</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \frac{d}{dt} E(t) = -\theta_1 E(t) )</td>
<td>( \theta_1 = 4.76 \times 10^{-1} )</td>
<td>1.295 ( \times 10^{-12} )</td>
<td>0.405</td>
<td>0.1936</td>
</tr>
<tr>
<td>( \frac{d}{dt} C(t) = \theta_1 E(t) - \theta_2 C(t) )</td>
<td>( \theta_2 = 5.76 \times 10^{-1} )</td>
<td>0.1412</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

An optimal control algorithm was subsequently developed for gene delivery to take into account the efficacy, toxicity, and cell division proposing an important model-based tool for making decisions under uncertainty, which is lacking for gene delivery systems. The proposed modelling and control approach allows for the simulation of siRNA delivery for in vivo conditions in order to compute the optimal delivery profile in the presence of different practical constraints. One of such constraints is on the time to take into account the cell multiplication so that the therapeutic effect is manifested before cell division takes place. Cell-doubling effect was addressed to develop a more realistic representation of model-based control of gene delivery that enables to predict the distribution of genetic materials in vivo and before cells divide. A constrained optimisation problem was formulated and solved while considering different cell division times to account for uncertainty in cell doubling time. Time profiles of optimal dosage infusion rate and optimal intracellular exposure of siRNAs were obtained in order to exert maximum therapeutic effects. It was also
demonstrated how these profiles can be affected by the trade-offs between toxicity and efficacy. The multi-objective optimisation framework was also set up to control the process system in the presence of other practical constraints such as incremental change in the infusion rate, $\Delta q(t)$, which could be constrained between certain lower and upper bounds. Incorporating of such process constraints is because of the practical limitations of gene delivery devices. In conclusion, the solution of the gene delivery optimal control problem has provided very interesting insights on what siRNA delivery profile might look like in a clinical setting. According to Petrocca and Lieberman (2011), continuous infusion might be used to prolong gene silencing. The results from this study also indicated that an optimal continuous infusion is superior to a single rapid bolus injection for achieving maximum gene silencing activity while preserving cell viability. Therefore, a promising platform for gene delivery systems was provided by model-based control technology, which can further assist in the optimisation of the process.

Moreover, the potential power of the developed models and control strategy was limited to intracellular barriers due to the lack of suitable experimental data. Model extensions require appropriate experimental analysis that describe spatiotemporal distribution of siRNAs in the extracellular matrix and intracellular environment. Another avenue that can be explored is to build a detailed mathematical model representing the biophysicochemical effects between nanocarriers and biological systems, in order to effectively investigate the intracellular reactions that occur in the cell.
Chapter 5.

5 MATHEMATICAL MODELLING OF GENE DELIVERY IN PATIENTS WITH HAEMOPHILIA B

5.1 Introduction

Having explored the nature and purpose of quantitative analysis of in vitro experimental data in the previous chapter, this chapter aims to further extend the methodology and develop a mathematical modelling approach, based on the available clinical data, for gene transfer of adeno-associated viral vectors in patients with haemophilia B.

Haemophilia B (HB) is a genetic bleeding disorder resulting from a deficiency or dysfunction of coagulation factor IX (FIX) caused by mutations in the gene that
encodes FIX (George et al., 2017; Ramaswamy et al., 2017). Although prophylactic therapy with factor IX protein concentrates improves clinical outcomes and reduces the frequency of spontaneous bleeding, but it requires frequent injections for the lifetime of patients due to the short half-life of the protein, resulting in an inconvenient and expensive (£140,000 per year per patient) treatment (Patel et al., 2014). Thus, various strategies have been investigated for the treatment of haemophilia B including the use of bioengineered coagulation factors (Powell et al., 2013), and gene-transfer therapy (Manno et al., 2006; Nathwani et al., 2014). Gene therapy is a potentially curative treatment option as it aims to restore, modify or enhance cellular functions through the introduction of a therapeutic gene into a target cell, which is demonstrated in the work by Nathwani et al. (2001; 2006; 2007; 2011(a); 2011(b); 2014). In the clinical trial conducted by Nathwani and colleagues, a single dose of a serotype-8-pseudotyped, self-complementary (sc) adeno-associated (AAV) vector expressing a codon-optimised version of the human factor IX (hFIXco) gene was infused in patients with severe HB whose FIX activity level is <1% of normal values (Nathwani et al., 2011). hFIXco transgene was synthesised and cloned downstream of a compact synthetic liver-specific promoter (LP1) to enable packaging into scAAV vectors (scAAV2/8-LP1-hFIXco) (Patel et al., 2014). The evaluation of safety and efficacy in HB patients, having had the peripheral-vein infusion of scAAV2/8-LP1-hFIXco, was reported in the work by Nathwani et al. (2014).

In this work, an integrated pharmacokinetic/pharmacodynamic model is developed using compartment modelling to describe the behaviour of scAAV2/8-LP1-hFIXco vectors in patients with HB, which will be then used for the initial dose selection.

### 5.2 Methods

#### 5.2.1 Clinical data

Nathwani et al. (2014) aimed to assess the efficacy and safety of factor IX gene therapy in patients with severe HB by evaluating the stability of transgene expression and monitoring the hepatocellular toxicity. The authors also reported the vector genomes in plasma, urine, stool, semen and saliva, which were collected from patients at regular intervals in order to assess vector shedding following systemic administration of scAAV2/8-LP1-hFIXco. The clinical data is used to build an integrated PK/PD model so as to be capable of providing a platform to guide initial
dose selection.

### 5.2.2 Pharmacokinetic Modelling

Physiologically based pharmacokinetic (PBPK) models, while being able to offer a more realistic picture of vector kinetics by modelling the real physiological space in the human body, are very complex and typically require more clinical data in more compartments for the validation of the models which are not readily available in clinical trials (Holz and Fahr, 2001). Therefore, a mechanistically lumped PK model was developed, based on the available clinical data, that comprised two compartments representing plasma (P) and body fluids (BF) (Figure 5.1 and Figure 5.2). Body fluids include urine, stool, semen, and saliva that were lumped into one compartment to represent the elimination process. This is because the parallel effluxes can be lumped into a single compartment (Holz and Fahr, 2001; Nestorov, 2003). Mathematically,

\[
\frac{dC_P}{dt} = -\theta_d C_P - \theta_{el,0} C_P = 0 
\]

\[
C_P(t = 0) = C_{P0} 
\]

\[
\frac{dC_{BF}}{dt} = \theta_{el,0} C_P - \theta_{el,1} C_{BF} 
\]

\[
C_{BF}(t = 0) = C_{BF0} 
\]

where \(C_P\) (vector genome/ml) and \(C_{BF}\) (vector genome/ml) are the vector concentrations in patient plasma and body fluids, respectively. \(\theta_d\) (day\(^{-1}\)) represents the distribution rate constant while \(\theta_{el,0}\) (day\(^{-1}\)) and \(\theta_{el,1}\) (day\(^{-1}\)) are the elimination rate constants.

The developed pharmacokinetic model serves as a platform for a quantitative evaluation of gene delivery. Equation 5.1 captures the rate of change of the vector concentration in patient plasma after a single intravenous infusion of vector. However, accounting for a continuous infusion into the PK model leads to a similar expression to Equation 4.3 in Chapter 4, thereby allowing for the computation of the optimal delivery profile:

\[
\frac{dC_P}{dt} = -\theta_d C_P - \theta_{el,0} C_P + q(t) 
\]
where \( q(t) \) (vector genome/ml/hr) is the vector infusion rate. Equation 5.3 involves the incorporation of the control variable to be optimised by a control algorithm, which will be the focus of future research.

5.2.3 Pharmacodynamic Modelling

Human factor IX (hFIX) is a coagulation protein, which is synthesised in the liver, and encoded in a gene located on the X chromosome (Howard et al., 2007; Tsang et al. 1988). Hepatocytes, which are the most common cells type in the liver, directly secrete factor IX into the bloodstream, where it circulates in an inactive form until needed in a response to an injury that damages the blood vessel wall. Since the liver is the primary site of FIX synthesis (Franchini et al., 2012), thus the site of action for scAAV2/8-LP1-hFlXco vectors is located in the liver compartment.

In order to develop a mathematical model, the plasma FIX activity has been considered as the pharmacological effect (response), which can be treated as an objective function to be maximised in the gene delivery optimal control problem. A physiological indirect response model with stimulation of factors controlling the response was thought to be appropriate to describe the vector pharmacodynamics. This is because of the time delay between the observed pharmacological effects and vector concentration in plasma as the pharmacological responses take time to be developed. The temporal displacement could be due to the vector tissue distribution phenomena to reach the site of action, liver. To this purpose, a dynamic model must be developed to link the vector concentration in the biophase or effect compartment to a response compartment. The effect compartment model, which is also known as the link model, can be considered as a first-order distribution model relating the vector concentration in plasma and the biophase using a first-order constant. Once the vector is transferred to the liver, a cascade of biological events may take place resulting in a functional response, which can be viewed as a link model. Schematic illustration of the integrated PK/PD model is shown in Figure 5.1.

While a more detailed representation of an integrated PK/PD approach can be developed by incorporating the liver compartment into the PK model, the model structure, which was developed and used in this work, had been simplified to only include the plasma and other body fluids compartments. This is due to a lack of available data as liver biopsies are required, as well as the identifiability issues and
numerical difficulties in the course of model assembly.

Figure 5.1: Schematic representation illustrating the relationship between kinetics and dynamics of the vector when considering the pharmacological response (plasma FIX coagulation activity level).

Considering the pharmacological analysis, the rate of change of the vector concentration in the effect (biophase) compartment, $C_{e,\text{FIX}}$ (vector genome/ml), can be modelled as:

$$\frac{dC_{e,\text{FIX}}}{dt} = \theta_{e,\text{FIX}} C_p - \theta_{in,\text{FIX}} C_{e,\text{FIX}}$$  \hspace{1cm} 5.4

where $C_p$ (vector genome/ml) is the concentration of vector in the plasma compartment of the pharmacokinetic model, linked to the effect compartment, with the first-order rate constant $\theta_{e,\text{FIX}}$ (day$^{-1}$).

The plasma FIX coagulation activity level, $R_{\text{FIX}}$ (%) of the normal value - IU/deciliter), which is of interest in our case, was reported by Nathwani et al. (2014), and is formulated as a function of the concentration in the effect compartment with the use of an effect-concentration model. The differential equation for the observed pharmacological effect, factor IX activity level, can be expressed as:

$$\frac{dR_{\text{FIX}}}{dt} = \theta_{in,\text{FIX}} E_{\text{FIX}} - \theta_{out,\text{FIX}} R_{\text{FIX}}$$  \hspace{1cm} 5.5

where the rate in and rate out of the response compartment are governed by
\( \theta_{in,\text{FIX}} (\text{day}^{-1}) \) and \( \theta_{out,\text{FIX}} (\text{day}^{-1}) \).

Note that the effect compartment model should be selected with an appropriate effect equation. In this study, the response is modelled by means of a linear transduction function in which the vector concentration is proportionally related to a pharmacological response. Therefore,

\[ E_{\text{FIX}} = C_{e,\text{FIX}} \]

5.2.4 Incorporating the Toxicological Model

The PD model may be extended to incorporate the toxicological responses that captures the liver toxicity, which was observed in the clinical study by Nathwani and colleagues as the primary endpoint of their study was the safety evaluation of the vector infusion at different doses. The level of serum alanine aminotransferase (ALT) over time were measured and reported by Nathwani et al. (2014) demonstrating the hepatocellular toxicity. ALT is an enzyme which is found in serum and organ tissues such as liver. The ALT level is the most widely used clinical biomarker of liver function, which may be elevated as a result of the leakage from the damaged hepatocytes into the plasma following hepatocellular injury (Washington and Van Hoosier, 2012).

\[ [\text{Vector}] \rightarrow \text{Dose} \rightarrow \text{Input} \rightarrow \theta_{d,0} \rightarrow \theta_{d-\text{ALT}} \rightarrow \text{Response} \]

\[ \theta_{in-\text{ALT}} \rightarrow E_{\text{ALT}} \rightarrow \text{Response} \rightarrow \theta_{out-\text{ALT}} \]

\[ \theta_{d,1} \rightarrow \text{Body Fluids} \rightarrow \text{Plasma} \rightarrow \text{Biophase} \rightarrow [\text{Vector}] \]

Figure 5.2: Schematic representation illustrating the relationship between kinetics and dynamics of the vector when considering the toxicological response (ALT level).
In this section, the structure of the PD model remained the same as in Section 5.2.3. Assuming an indirect response model with stimulation of factors controlling the toxicological response (Figure 5.2), the rate of change of the vector concentration in the effect (biophase) compartment, $C_{e,ALT}$ (vector genome/ml), can be modelled as:

$$\frac{dC_{e,ALT}}{dt} = \theta_{e,ALT} C_P - \theta_{in,ALT} C_{e,ALT}$$

where $C_P$ (vector genome/ml) is the concentration of vector in the plasma compartment of the pharmacokinetic model, linked to the effect compartment, with the first-order rate constant $\theta_{e,ALT}$ (day$^{-1}$).

The ALT level, $R_{ALT}$ (IU/liter), is formulated as a function of the concentration in the effect compartment with the use of an effect-concentration model:

$$\frac{dR_{ALT}}{dt} = \theta_{in,ALT} E_{ALT} - \theta_{out,ALT} R_{ALT}$$

$$E_{ALT} = C_{e,ALT}$$

where the rate in and rate out of the response compartment are governed by $\theta_{in,ALT}$ (day$^{-1}$) and $\theta_{out,ALT}$ (day$^{-1}$).

### 5.3 Results

The proposed modelling framework will be evaluated for three patients with severe HB who had received intermediate dose of vector, $6 \times 10^{11}$ vector genomes [vg] per kilogram [kg] of body weight, (patient 4); and high dose of vector, $2 \times 10^{12}$ vg per kg, (patients 6 and 9). Table 5.1 summarises the key characteristics of the patients.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Vector Dose, $6 \times 10^{11}$ vg/kg</th>
<th>Vector Dose, $2 \times 10^{12}$ vg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patient 4</td>
<td>Patient 6</td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td>Male</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>29</td>
<td>27</td>
</tr>
<tr>
<td>Factor IX prophylaxis</td>
<td>Once weekly</td>
<td>Three times weekly weekly</td>
</tr>
<tr>
<td>HIV status</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Hepatitis C status</td>
<td>Negative</td>
<td>Negative</td>
</tr>
</tbody>
</table>
The results obtained from this study will be presented in two parts. First, the results of the parameter estimation problem will be discussed in Section 5.3.1. Then, a number of dynamic simulations will be presented in Section 5.3.2 for initial dose selection.

### 5.3.1 Parameter Estimation

Having the PK/PD model and clinical data, the parameter estimation problem was formulated as an optimisation problem, and solved using the analytical solution of the PK and PD models. Since the spread of values in the PK clinical data set is large, the PK parameter estimation problem was performed using both absolute and scaled objective functions. The full set of model parameters and state variables are listed in Table 5.2.

**Table 5.2:** Model parameters and state variables of the PK/PD model.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_P )</td>
<td>Vector concentration in the plasma compartment</td>
<td>vg/ml</td>
</tr>
<tr>
<td>( C_{BF} )</td>
<td>Vector concentration in the body fluids compartment</td>
<td>vg/ml</td>
</tr>
<tr>
<td>( C_{e_{FIX}} )</td>
<td>Vector concentration in the biophase (effect) compartment when considering the pharmacological response (FIX coagulation activity level)</td>
<td>vg/ml</td>
</tr>
<tr>
<td>( C_{e_{ALT}} )</td>
<td>Vector concentration in the biophase (effect) compartment when considering the toxicological response (ALT level)</td>
<td>vg/ml</td>
</tr>
<tr>
<td>( R_{FIX} )</td>
<td>Plasma factor IX coagulation activity level</td>
<td>IU/dl</td>
</tr>
<tr>
<td>( R_{ALT} )</td>
<td>ALT level</td>
<td>IU/L</td>
</tr>
<tr>
<td>( \theta_d )</td>
<td>Distribution rate constant</td>
<td>day(^{-1})</td>
</tr>
<tr>
<td>( \theta_{el0} )</td>
<td>Elimination rate constant</td>
<td>day(^{-1})</td>
</tr>
<tr>
<td>( \theta_{el1} )</td>
<td>Elimination rate constant</td>
<td>day(^{-1})</td>
</tr>
<tr>
<td>( \theta_{e_{FIX}} )</td>
<td>Rate constant linking a kinetic model and a dynamic model when considering the pharmacological response (FIX coagulation activity level)</td>
<td>day(^{-1})</td>
</tr>
</tbody>
</table>
Chapter 5

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\theta_{e,\text{ALT}}$</td>
<td>Rate constant linking a kinetic model and a dynamic model when considering the toxicological response (ALT level)</td>
<td>day$^{-1}$</td>
</tr>
<tr>
<td>$\theta_{\text{in,FIX}}$</td>
<td>The rate in of the pharmacological response compartment ($R_{\text{FIX}}$)</td>
<td>day$^{-1}$</td>
</tr>
<tr>
<td>$\theta_{\text{out,FIX}}$</td>
<td>The rate out of the pharmacological response compartment ($R_{\text{FIX}}$)</td>
<td>day$^{-1}$</td>
</tr>
<tr>
<td>$\theta_{\text{in,ALT}}$</td>
<td>The rate in of the toxicological response compartment ($R_{\text{ALT}}$)</td>
<td>day$^{-1}$</td>
</tr>
<tr>
<td>$\theta_{\text{out,ALT}}$</td>
<td>The rate out of the toxicological response compartment ($R_{\text{ALT}}$)</td>
<td>day$^{-1}$</td>
</tr>
</tbody>
</table>

**Err$_{\text{absolute}}$** Absolute objective function

**Err$_{\text{scaled}}$** Scaled objective function

$\Psi_k$ The vector of the state variables in compartment $k$

$\tilde{\Psi}_k$ The vector of the observed clinical data in compartment $k$

$\theta$ The vector of the model parameters

The generic mathematical formulation of the parameter estimation problem is as follows:

$$\text{Err}_{\text{absolute}} = \min_{\theta, \Psi(t)} \sum_{p \in P} \sum_{k \in K} \left( \Psi_k(t_p) - \tilde{\Psi}_k(t_p) \right)^2$$

or

$$\text{Err}_{\text{scaled}} = \min_{\theta, \Psi(t)} \sum_{p \in P} \sum_{k \in K} \left( \frac{\Psi_k(t_p) - \tilde{\Psi}_k(t_p)}{\Psi_k(t_p)} \right)^2$$

subject to the analytical solutions of the PK/PD model:

$$C_p(t) = C_{p0} \, e^{(-\theta_d - \theta_{el0}) \, t}$$
Chapter 5

\[ C_{BF}(t) = C_{BF0} e^{-\theta_{el.1} t} + \frac{e^{-\theta_{el.1} t}(-1 + e^{(-\theta_{d}-\theta_{el.0}) t + \theta_{el.1} t}) \theta_{el.0} C_{P0}}{-\theta_{d} - \theta_{el.0} + \theta_{el.1}} \]

\[ C_{e,\text{FIX}}(t) = C_{e,\text{FIX} 0} e^{-\theta_{in,\text{FIX}} t} \]

\[ + \frac{e^{-\theta_{in,\text{FIX}} t}(-1 + e^{(-\theta_{d}-\theta_{el.0}) t + \theta_{in,\text{FIX}} t}) \theta_{in,\text{FIX}} C_{P0}}{-\theta_{d} - \theta_{el.0} + \theta_{in,\text{FIX}}} \]

\[ R_{\text{FIX}}(t) = \frac{e^{-\theta_{in,\text{FIX}} t - \theta_{out,\text{FIX}} t}(e^{\theta_{in,\text{FIX}} t} - e^{\theta_{out,\text{FIX}} t}) \theta_{in,\text{FIX}} C_{e,\text{FIX} 0}}{\theta_{in,\text{FIX}} \theta_{out,\text{FIX}}} \]

\[ + R_{\text{FIX} 0} e^{-\theta_{out,\text{FIX}} t} \]

\[ + \left( e^{-\theta_{in,\text{FIX}} t - \theta_{out,\text{FIX}} t} \theta_{e,\text{FIX}} \theta_{in,\text{FIX}} \left( -e^{\theta_{in,\text{FIX}} t} \theta_{in,\text{FIX}} + e^{\theta_{out,\text{FIX}} t} \theta_{out,\text{FIX}} \right) \right) \]

\[ = \frac{e^{\theta_{in,\text{FIX}} t} + \theta_{out,\text{FIX}} t + (-\theta_{d}-\theta_{el.0}) t \theta_{in,\text{FIX}} + e^{\theta_{out,\text{FIX}} t} \theta_{out,\text{FIX}}}{\theta_{in,\text{FIX}} \theta_{out,\text{FIX}}} \]

\[ - e^{\theta_{out,\text{FIX}} t} \theta_{d} + e^{\theta_{in,\text{FIX}} t} \theta_{el.0} \theta_{out,\text{FIX}} \left( \theta_{in,\text{FIX}} - \theta_{d} - \theta_{el.0} \right) \left( \theta_{out,\text{FIX}} - \theta_{d} \right) \]

\[ \times \left( \theta_{in,\text{FIX}} - \theta_{out,\text{FIX}} \right) \left( \theta_{in,\text{FIX}} - \theta_{d} - \theta_{el.0} \right) \left( \theta_{out,\text{FIX}} - \theta_{d} \right) \]

\[ - \theta_{el.0} \right) \]

\[ C_{e,\text{ALT}}(t) = C_{e,\text{ALT} 0} e^{-\theta_{in,\text{ALT}} t} \]

\[ + \frac{e^{-\theta_{in,\text{ALT}} t}(-1 + e^{(-\theta_{d}-\theta_{el.0}) t + \theta_{in,\text{ALT}} t}) \theta_{in,\text{ALT}} C_{P0}}{-\theta_{d} - \theta_{el.0} + \theta_{in,\text{ALT}}} \]
\[ R_{ALT}(t) = \frac{e^{-\theta_{in,ALT} t - \theta_{out,ALT} t} (e^{\theta_{in,ALT} t} - e^{\theta_{out,ALT} t})}{\theta_{in,ALT} - \theta_{out,ALT}} \theta_{in,ALT} C_{e,ALT} 0 + R_{ALT} 0 e^{-\theta_{out,ALT} t} \]

\[ + \left( e^{-\theta_{in,ALT} t - \theta_{out,ALT} t} \theta_{e,ALT} \theta_{in,ALT} \left( -e^{\theta_{in,ALT} t} \theta_{in,ALT} \right) \right. \]

\[ + e^{\theta_{in,ALT} t + \theta_{out,ALT} t + (-\theta_{d} - \theta_{el,0}) t} \theta_{in,ALT} + e^{\theta_{out,ALT} t} \theta_{out,ALT} \]

\[ - e^{\theta_{in,ALT} t + \theta_{out,ALT} t + (-\theta_{d} - \theta_{el,0}) t} \theta_{out,ALT} + e^{\theta_{in,ALT} t} \theta_{d} \]

\[ - e^{\theta_{out,ALT} t} \theta_{d} + e^{\theta_{in,ALT} t} \theta_{e,0} - e^{\theta_{out,ALT} t} \theta_{el,0} \]  

\[ \times C_{p0} \]

\[ / \left( \theta_{in,ALT} - \theta_{out,ALT} \right) \left( \theta_{in,ALT} - \theta_{d} - \theta_{el,0} \right) \left( \theta_{out,ALT} - \theta_{d} \right) \]

where \( \Psi_k \) is the vector of the state variables in compartment \( k \), \( \Psi_k \) represents the vector of the observed clinical data in compartment \( k \), and \( \Theta \) is the vector of the model parameters to be estimated such that the error, \( Err \), between the measured data and the model predictions is minimised. \( Err_{\text{absolute}} \) and \( Err_{\text{scaled}} \) denote the absolute and scaled objective functions, respectively.

To carry out parameter estimation for the system, first, PK/PD parameters were estimated individually for each patient, which could be useful for the development of personalised gene therapy. Then, each PK and PD parameters were estimated for all patients simultaneously, which were used for the initial dose selection aiming at predicting the physiological response of a patient to a dose of vector. For individually estimated PK/PD parameters, the analysis was dependent on the initial vector concentration, whereas the simultaneous parameter estimation was dose-dependent. Table 5.3 and Table 5.4 summarise the parameter estimation results for individually and simultaneously estimated parameters. The estimated parameter values were then used for dynamic simulations using OCFE, which were carried out for the validation of the model, with a view to pave the way for control in future work. Note that the model parameters are specific to a patient and may vary between patients.
(inter-patient) and also within individual patients (intra-patient). There are different factors that affect inter- and intra-patient variability, such as gender, age, body weight, health condition and activity levels.

Table 5.3: Estimated PK/PD model parameters, individually for each patient.

<table>
<thead>
<tr>
<th>Patient 4 (P.4)</th>
<th>Estimated parameters (day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PK Model</strong></td>
<td></td>
</tr>
<tr>
<td>Absolute OBJ*</td>
<td>$\theta_d = 1.5710559$</td>
</tr>
<tr>
<td></td>
<td>$\theta_{e,lo} = 1.0506940$</td>
</tr>
<tr>
<td>Scaled OBJ*</td>
<td>$\theta_d = 2.5971076$</td>
</tr>
<tr>
<td></td>
<td>$\theta_{e,lo} = 0.0247028$</td>
</tr>
<tr>
<td><strong>PD Model</strong></td>
<td></td>
</tr>
<tr>
<td>FIX</td>
<td>$\theta_{e,fix} = 9.7701316$</td>
</tr>
<tr>
<td></td>
<td>$\theta_{in,fix} = 0.0016288$</td>
</tr>
<tr>
<td>ALT</td>
<td>$\theta_{e,alt} = 18.4752261$</td>
</tr>
<tr>
<td></td>
<td>$\theta_{in,alt} = 0.0005428$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patient 6 (P.6)</th>
<th>Estimated parameters (day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PK Model</strong></td>
<td></td>
</tr>
<tr>
<td>Absolute OBJ*</td>
<td>$\theta_d = 2.1140705$</td>
</tr>
<tr>
<td></td>
<td>$\theta_{e,lo} = 0.0073093$</td>
</tr>
<tr>
<td>Scaled OBJ*</td>
<td>$\theta_d = 2.0194024$</td>
</tr>
<tr>
<td></td>
<td>$\theta_{e,lo} = 0.0910754$</td>
</tr>
<tr>
<td><strong>PD Model</strong></td>
<td></td>
</tr>
<tr>
<td>FIX</td>
<td>$\theta_{e,fix} = 21.1668725$</td>
</tr>
<tr>
<td></td>
<td>$\theta_{in,fix} = 0.0003748$</td>
</tr>
<tr>
<td>ALT</td>
<td>$\theta_{e,alt} = 0.3656878$</td>
</tr>
<tr>
<td></td>
<td>$\theta_{in,alt} = 0.0028681$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patient 9 (P.9)</th>
<th>Estimated parameters (day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PK Model</strong></td>
<td></td>
</tr>
<tr>
<td>Absolute OBJ*</td>
<td>$\theta_d = 0.1593991$</td>
</tr>
<tr>
<td></td>
<td>$\theta_{e,lo} = 1.1246204$</td>
</tr>
<tr>
<td>Scaled OBJ*</td>
<td>$\theta_d = 0.9911402$</td>
</tr>
<tr>
<td></td>
<td>$\theta_{e,lo} = 0.3439847$</td>
</tr>
<tr>
<td><strong>PD Model</strong></td>
<td></td>
</tr>
<tr>
<td>FIX</td>
<td>$\theta_{e,fix} = 2.0086934$</td>
</tr>
<tr>
<td></td>
<td>$\theta_{in,fix} = 0.0012088$</td>
</tr>
<tr>
<td>ALT</td>
<td>$\theta_{e,alt} = 0.64510203$</td>
</tr>
<tr>
<td></td>
<td>$\theta_{in,alt} = 0.0010856$</td>
</tr>
</tbody>
</table>

Table 5.4: Estimated PK/PD model parameters, for all patients simultaneously.

<table>
<thead>
<tr>
<th>Patients 4, 6, and 9 (P.4-6-9)</th>
<th>Estimated parameters (day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PK Model</strong></td>
<td></td>
</tr>
<tr>
<td>Absolute OBJ*</td>
<td>$\theta_d = 1.5511141$</td>
</tr>
<tr>
<td></td>
<td>$\theta_{e,lo} = 0.4723049$</td>
</tr>
<tr>
<td>Scaled OBJ*</td>
<td>$\theta_d = 7.1957447$</td>
</tr>
<tr>
<td></td>
<td>$\theta_{e,lo} = 2.0910047$</td>
</tr>
<tr>
<td><strong>PD Model</strong></td>
<td></td>
</tr>
<tr>
<td>FIX</td>
<td>$\theta_{e,fix} = 11.2140501$</td>
</tr>
<tr>
<td></td>
<td>$\theta_{in,fix} = 0.0005731$</td>
</tr>
<tr>
<td>ALT</td>
<td>$\theta_{e,alt} = 0.6582939$</td>
</tr>
<tr>
<td></td>
<td>$\theta_{in,alt} = 0.0007284$</td>
</tr>
</tbody>
</table>

* – Solved the parameter estimation problem using an absolute objective function (Equation 5.10).

▲ – Solved the parameter estimation problem using a scaled objective function (Equation 5.11).

In order to visualise the variance between the estimated PK/PD parameters across different patients, the results are also graphically shown in Figure 5.3. Note that in the following figure, P.4, P.6, and P.9 refer to Patient 4, Patient 6, and Patient 9.
respectively, where the PK and PD parameters were estimated individually for each patient. However, P.4-6-9 refers to the population modelling approach in which each PK and PD parameters were estimated for all patients simultaneously.

![Figure 5.3: Estimated PK/PD parameters across different patients.](image)
It is important to note here that the estimated model parameters could vary for different initial guesses used for the parameter estimation problem. Difficulties arise from both the existence of local minima and non-identifiability (Degasperi et al., 2017). The solver may find different local minima when started from different starting points due to the non-convexity of the objective function. This may be overcome by using global optimisation-based algorithms. However, the identifiability issue is concerned with the theoretical existence of unique solutions to the parameter estimation problem. Hence, there are various sets of parameter values that fit the clinical data equally well. Different strategies, such as model reformulation, model reduction, or generating additional clinical data can be used to overcome the identifiability problem (Degasperi et al., 2017). In Figure 5.3, the variability of the estimated model parameters across different patients could be associated with the inter-patient variability, suggesting that the personalised gene therapy using an individual modelling approach would make more sense because the pharmacokinetics and pharmacodynamics of the vector can be variable between patients. However, to gain more insights into the process, both the individual modelling approach (solving the parameter estimation problem for each patient individually) and the population modelling approach (solving the parameter estimation problem for all patients simultaneously) were considered in the present work.

The results obtained from the PK/PD analysis using an individual modelling are shown in Figure 5.4, Figure 5.5, and Figure 5.6, while the results illustrated in Figure 5.7, Figure 5.8, and Figure 5.9 present the PK/PD analysis using a population modelling. The parameter estimation and the simulation results obtained from the work, have been qualitatively verified by using the compartmental modelling approach. As can be seen from the following figures, the dynamic simulations agreed closely with the parameter estimation results, and the model predictions are in good accordance with the clinical data. However, depending on the type of the objective function and the choice of individual modelling approach or population modelling approach, various results of the study highlighted several feasible configurations of the system. Such considerations were taken into account to aid decision making for further research. The values of the objective function obtained for each case study are reported in Table 5.5 and Table 5.6, which give an indication of the solution accuracy. The results will be discussed more later.
Chapter 5

**Patient 4**

(a) Plasma

(b) Body Fluids

**Patient 6**

(c) Plasma

(d) Body Fluids

**Patient 9**

(e) Plasma

(f) Body Fluids

![Graphs showing pharmacokinetic analysis](image)

- Clinical data
- Parameter estimation using analytical solution
- Model simulation for estimated parameters using OCFE

**Figure 5.4:** Pharmacokinetic Analysis, individually for each patient – Comparison of the PK model predictions (using an absolute objective function) with the clinical data.
Figure 5.5: Pharmacokinetic Analysis, individually for each patient – Comparison of the PK model predictions (using a scaled objective function) with the clinical data.
Figure 5.6: Pharmacodynamic Analysis, individually for each patient – Comparison of the PD model predictions (using an absolute objective function) with the clinical data.
Figure 5.7: Pharmacokinetic Analysis, for all patients simultaneously – Comparison of the PK model predictions (using an absolute objective function) with the clinical data.
Chapter 5

Patient 4

(a) Plasma

(b) Body Fluids

Patient 6

(c) Plasma

(d) Body Fluids

Patient 9

(e) Plasma

(f) Body Fluids

Figure 5.8: Pharmacokinetic Analysis, for all patients simultaneously – Comparison of the PK model predictions (using a scaled objective function) with the clinical data.
Figure 5.9: Pharmacodynamic Analysis, for all patients simultaneously – Comparison of the PD model predictions (using an absolute objective function) with the clinical data.
Chapter 5

Table 5.5: Computational demand for the individual modelling approach.

<table>
<thead>
<tr>
<th>Patient 4</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PK Model</strong></td>
<td>Objective function values</td>
<td>Corresponding figures</td>
</tr>
<tr>
<td></td>
<td>$\text{Err}_{\text{absolute}} = 1.2013 \times 10^{-5}$</td>
<td>Figure 5.4 (a) and (b)</td>
</tr>
<tr>
<td></td>
<td>$\text{Err}_{\text{scaled}} = 1.667 \times 10^{-16}$</td>
<td>Figure 5.5 (a) and (b)</td>
</tr>
<tr>
<td><strong>PD Model - FIX</strong></td>
<td>$\text{Err}_{\text{absolute}} = 52.140$</td>
<td>Figure 5.6 (a)</td>
</tr>
<tr>
<td><strong>PD Model - ALT</strong></td>
<td>$\text{Err}_{\text{absolute}} = 1399.890$</td>
<td>Figure 5.6 (b)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patient 6</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PK Model</strong></td>
<td>Objective function values</td>
<td>Corresponding figures</td>
</tr>
<tr>
<td></td>
<td>$\text{Err}_{\text{absolute}} = 1.0396 \times 10^{-5}$</td>
<td>Figure 5.4 (c) and (d)</td>
</tr>
<tr>
<td></td>
<td>$\text{Err}_{\text{scaled}} = 2.990$</td>
<td>Figure 5.5 (c) and (d)</td>
</tr>
<tr>
<td><strong>PD Model - FIX</strong></td>
<td>$\text{Err}_{\text{absolute}} = 200.021$</td>
<td>Figure 5.6 (c)</td>
</tr>
<tr>
<td><strong>PD Model - ALT</strong></td>
<td>$\text{Err}_{\text{absolute}} = 799.967$</td>
<td>Figure 5.6 (d)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patient 9</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PK Model</strong></td>
<td>Objective function values</td>
<td>Corresponding figures</td>
</tr>
<tr>
<td></td>
<td>$\text{Err}_{\text{absolute}} = 3 \times 10^{-1}$</td>
<td>Figure 5.4 (e) and (f)</td>
</tr>
<tr>
<td></td>
<td>$\text{Err}_{\text{scaled}} = 2.997$</td>
<td>Figure 5.5 (e) and (f)</td>
</tr>
<tr>
<td><strong>PD Model - FIX</strong></td>
<td>$\text{Err}_{\text{absolute}} = 37.134$</td>
<td>Figure 5.6 (e)</td>
</tr>
<tr>
<td><strong>PD Model - ALT</strong></td>
<td>$\text{Err}_{\text{absolute}} = 187.068$</td>
<td>Figure 5.6 (f)</td>
</tr>
</tbody>
</table>

Table 5.6: Computational demand for the population modelling approach.

<table>
<thead>
<tr>
<th>Patients 4, 6, and 9</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PK Model</strong></td>
<td>Objective function values</td>
<td>Corresponding figures</td>
</tr>
<tr>
<td></td>
<td>$\text{Err}_{\text{absolute}} = 1481.198$</td>
<td>Figure 5.7</td>
</tr>
<tr>
<td></td>
<td>$\text{Err}_{\text{scaled}} = 10.270$</td>
<td>Figure 5.8</td>
</tr>
<tr>
<td><strong>PD Model - FIX</strong></td>
<td>$\text{Err}_{\text{absolute}} = 1011.102$</td>
<td>Figure 5.9 (a), (c), and (e)</td>
</tr>
<tr>
<td><strong>PD Model - ALT</strong></td>
<td>$\text{Err}_{\text{absolute}} = 4167.984$</td>
<td>Figure 5.9 (b), (d), and (f)</td>
</tr>
</tbody>
</table>

According to the results, the objective function values observed for the PD parameter estimation are much higher than those obtained for the PK parameter estimation. This is because of the extensive PD data set and the widespread existence of fluctuations in the PD clinical data. Another potential contributor is the existence of hypothetical effect compartment that acts as a link between the PK and PD models. However, the analysis shows that a good match is obtained between the clinical data and the model predictions.

The pharmacokinetic analysis in this work demonstrates how the overall performance
of the PK parameter estimation problem depends on the optimisation algorithms and the objective functions. Making such comparisons between an absolute objective function and a scaled objective function lead to the fact that using a scaling factor may cause an algorithm to determine a different optimal solution. The absolute and scaled objective function values vary with no observable trend. Hence, based on a trade-off between the objective function values and the simulation results, a decision is made to use a set of parameters for subsequent computational studies.

5.3.2 Initial Dose Selection

This section aims to explore how the simulation-based modelling approach can assist in the initial dose selection. In this work, the initial doses used for the simulations are calculated based on the following assumptions: (i) the average plasma volume is 50 ml/kg (Yiengst and Shock, 1962); and (ii) there is a linear relationship between the dose administered (after conversion from vg/kg to vg/ml) and the initial vector concentration in plasma. Linear regression is one of the most commonly used techniques to investigate the relationship between two quantitative variables (Bewick et al., 2003). Therefore, a linear regression analysis was carried out to determine the equation of the regression line, which is as follows and shown in Figure 5.10: Initial vector concentration in plasma = $5 \times 10^{-5} \times $Dose − 287000.

![Figure 5.10: Linear regression curve between the dose administered and the initial vector concentration in plasma.](image)

For comparison purposes, the dynamic simulations were carried out for different time periods and for various initial bolus doses. The PK/PD profiles are shown in Figure 5.11, Figure 5.12, Figure 5.13, and Figure 5.14.
Figure 5.11: Population pharmacokinetic and pharmacodynamic analysis over a period of 30 days for different initial bolus doses.

Figure 5.12: Population pharmacokinetic and pharmacodynamic analysis over a period of 60 days for different initial bolus doses.
Chapter 5

Figure 5.13: Population pharmacokinetic and pharmacodynamic analysis over a period of 90 days for different initial bolus doses.

Figure 5.14: Population pharmacokinetic and pharmacodynamic analysis over a period of 3 years for different initial bolus doses.
Chapter 5

As can be seen in Figure 5.11b, Figure 5.12b, Figure 5.13b, and Figure 5.14b, the vector is expected to be eliminated from the body within 10 days after administration. From the simulation results (Figure 5.11, Figure 5.12, Figure 5.13, and Figure 5.14), it can be seen that the increase in both factor IX activity and ALT level is dose-dependent, which is consistent with the work by Nathwani et al. (2014). The results show that maximum efficacy can be reached within 3 months after infusion. However, ALT levels are also increased consistently, especially in higher dose cohorts, which subsequently leads to a relative reduction in factor IX levels (about 55% reduction). According to Nathwani et al. (2014), the increase in the ALT level is associated with a decline in factor IX activity levels, suggesting a loss of transduced hepatocytes.

5.4 Conclusions

In this chapter, a mathematical modelling approach was developed for gene transfer of adeno-associated viral vectors in patients with haemophilia B. A number of case studies were analysed for three patients of the clinical study. The model-based platform discussed in this chapter incorporates the pharmacokinetics and pharmacodynamics of the scAAV2/8-LP1-hFIXco vectors. The PK/PD model parameters were estimated using the analytical solution of the model, individually for each patient in a dose-independent manner, and for all patients simultaneously in a dose-dependent manner. A number of dynamic simulations were also carried out using OCFE for the validation of the model, demonstrating the simulation results are comparable to that obtained from parameter estimation. The simulation-based PK/PD modelling approach was then used for the initial dose selection to provide clinicians with better tools to make the decision-making process simpler for designing more effective treatment plans, which can be tailored to maximise efficacy while minimising toxicity for individual patients.
6 PARAMETER ESTIMATION OF PARTIAL DIFFERENTIAL EQUATIONS USING ARTIFICIAL NEURAL NETWORK

6.1 Introduction

Mathematical models of biological systems can be classified as either lumped or distributed systems (Bonate, 2011). In a lumped system, different organs are lumped into single groups. Compart ment modelling approach, which was used in Chapters 4 and 5, is the classic example of such systems and is a well-established methodology that has been validated mathematically and clinically. This approach is the most efficient computational treatment of a cell, which is often used to describe transport
and reaction in biological systems (Dreij et al., 2011; Holz and Fahr, 2001; Jacquez, 1996). In compartment modelling, the diffusion is assumed to be very fast compared to the reaction rates such that the concentration of genetic materials is constant throughout the compartment, so the intra- and extracellular domains are treated as well-stirred compartments. The advantage of using a compartment modelling approach includes reducing the model complexity and thus the computational cost, which makes it amenable for control purposes (Dreij et al., 2011; Parker and Doyle, 2001). While a lot of important work has been done in compartment modelling, there are more potential areas which are yet to be explored adequately as this technique approximates all spatial and transport processes by kinetic equations.

In a distributed system, the spatial aspects of the system can be built into the model. Mathematical models of a distributed system are typically complex, often requiring the numerical solution of partial differential equations (PDEs), which few pharmacokineticists are familiar with and few software packages are equipped to solve (Bonate, 2011). However, in order to emphasise the spatial and physical aspects of intracellular trafficking of genetic materials and to provide a realistic representation of cell geometry, a more sophisticated model using PDEs may be required (Dinh et al., 2007). A mathematical model including the reaction-diffusion system was developed by Chaudhry (2012) to understand the cellular behaviour of reactive toxic chemical compounds in mammalian cells. The author proposed a Non-Standard Compartment Model in which the system consists of both ODEs and PDEs representing the reaction and diffusion mechanisms in- and outside the cell. The membranes including cell and nuclear membranes were considered as spatially distributed sub-domains, where the remaining sub-domains, such as extracellular, cytoplasm and nucleus were treated as well-stirred compartments. The numerical results obtained from the model were reported to be in good agreement with the in vitro cell experimental results (Chaudhry, 2012).

Motivated by the work of Chaudhry (2012), a schematic diagram is proposed for a gene delivery system (Figure 6.1) representing the compartmental and spatially distributed system. In Figure 6.1, cytoplasm and nucleus are well-stirred compartments that can be modelled as biochemical reactions using ODEs, whereas the nuclear membrane is the spatially distributed compartment that can be modelled using PDEs.
Figure 6.1: Compartmental and spatially distributed system with three compartments. A simple model adapted from Chaudhry (2012).

A general mathematical formulation of the diffusion model for a gene delivery system is presented as follows:

\[
\frac{\partial}{\partial t} C(t,x) = D \frac{\partial^2}{\partial x^2} C(t,x)
\]

subject to the associated initial and boundary conditions, where \( C(t,x) \) (mass / volume) represents the concentration of the diffusing gene in the membrane, which is a function of space and time, and \( D \) (area / time) denotes the diffusion coefficient.

Although a system of reaction-diffusion partial differential equations is able to evaluate the spatiotemporal distribution of therapeutic genes in the extra- and intracellular environments; however, one of the major challenges associated with the development of mathematical models for these types of systems is the availability of proper experimental data. Therefore, further development of PDE models for gene delivery systems remains to be seen. Furthermore, the cellular geometry plays a more consequential role in diffusion through the membranes (Chaudhry, 2012), and according to Dreij et al., (2011), the geometry of the cellular compartments could be very complex. Hence, the first step to construct a high-fidelity spatial-temporal mathematical model (PDE) for complex cellular architectures is to develop an efficient and reliable method for solving parameter estimation problems to account for domains with irregular boundaries.

The research work presented in this chapter aims at developing a novel meshless parameter estimation framework for a system of partial differential equations using simultaneous optimisation and solution strategy based on the neural network
approximations. Since the approximation capabilities of the feedforward artificial neural networks (ANNs) have been widely acknowledged (Hornik et al., 1989; Lagaris et al., 1998; Leshno et al., 1993), and the ANN-based methodology for parameter estimation was successfully examined for ODE systems (Dua, 2011; Dua and Dua, 2012); therefore, it is of interest to consider this meshless scheme as a candidate for the estimation of parameters in partial differential equations. In this work, the PDE models to be treated consist of linear and nonlinear partial differential equations, with Dirichlet and Neumann boundary conditions (BCs), considering both regular and irregular boundaries (Lagaris et al., 1998, 2000).

A neural network based model has various advantages over the standard numerical approximations (Yadav et al., 2015), such as:

i. The ANN-based solution of a differential equation is in a closed analytic form that is differentiable; hence, can be easily used in any subsequent computations, while other numerical methods offer a discrete solution or with limited differentiability;

ii. The ANN-based method can be implemented in systems defined on either simple orthogonal box boundaries or on any arbitrarily complex geometrical shape boundaries;

iii. Highly non-linear functions can be treated by using the ANN formulation.

This chapter focuses on testing the applicability of neural networks for estimating the process parameters while simultaneously providing a solution to the system of PDEs representing the process. A description of the parameter estimation methodology is first presented in general terms, along with details about relevant models for the approximation of the solution. The capability of the proposed methodology is then demonstrated with five numerical problems. Finally, a summary of the problems and the solutions obtained concludes the chapter.

6.2 Parameter Estimation Methodology

6.2.1 Description of the Method

The proposed approach in this part of the thesis will be illustrated in terms of the partial differential equations under the following assumptions, (i) the PDE model
structure of the system to be investigated is pre-selected and known, (ii) the system is identifiable, and (iii) the measured data (experimental observations) are available. Therefore, the main objective is to compute the unknown model parameters while simultaneously providing a solution to the system of PDEs.

Using the Least Squares (LS) objective function, the parameter estimation problem is formulated as follows:

$$\min_{\theta, \Psi(x)} \text{Err}_{PE} = \sum_{p \in P} \{\Psi(x^p) - \Psi(x^p)\}^2$$  \hspace{1cm} \text{6.2}

subject to the PDE model taking the form of:

$$J(\partial^s \Psi, \partial^{s-1} \Psi, \ldots, \partial \Psi, \Psi, x) = F_k(\Psi(x), \theta, x)$$  \hspace{1cm} \text{6.3}

and associated boundary conditions, where $\Psi := (\Psi_1(x), \ldots, \Psi_k(x)) \in \mathbb{R}^{n_\Psi}; n_\Psi \in \mathbb{N}$, denotes the vector of $k$ unknown functions of state variables in the given system of PDEs. It is assumed that the definition domain, $x := (x_1, \ldots, x_m) \in \mathbb{R}^{n_x}; n_x \in \mathbb{N}$, and the right-hand side of the equations, $F_k(\Psi(x), \theta, x)$, have been given. If the time is included as one of the independent variables, it can be identified as the zeroth variable, $x_0 = t$. Note that the order of the differential equation is determined by $s$. $\Psi(x^p)$ represents the experimental measurements of the state variables at data points $x^p; p \in P \subseteq \mathbb{N}$, and $\theta$ is the vector of model parameters to be estimated such that the error, $\text{Err}_{PE}$, between the measured data and the model predictions is minimised.

The methodology proposed in this work involves two main steps: first, approximating the solution by a trial solution, and second, incorporating the boundary conditions within the trial solution, as explained next.

Let $\Psi_{k}^{ANN}(x)$ denotes the trial solution. The ANN approximation of the model is formulated as follows, and incorporated into the parameter estimation problem:

$$\sum_{p \in P} \sum_{k \in K} \{J(\partial^s \Psi_{k}^{ANN}, \partial^{s-1} \Psi_{k}^{ANN}, \ldots, \partial \Psi_{k}^{ANN}, \Psi_{k}^{ANN}, x^p) - F_k(\Psi(x^p), \theta, x^p)\}^2 \leq \varepsilon$$  \hspace{1cm} \text{6.4}

In the proposed approach, a trial form of the solution (or the neural network approximation of the solution), $\Psi^{ANN}$, is chosen (by construction) such that the
initial/boundary conditions of the differential equation model are satisfied. The trial solution involves a sum of two terms:

$$\Psi^{ANN}(x) = A(x) + F(x, N(x))$$ \hspace{1cm} (6.5)

where the first term, $A(x)$, is independent of adjustable parameters so as to satisfy the BCs, while the term, $F$, is constructed to employ a feedforward neural network involving adjustable parameters such as weights and biases to deal with the minimisation problem. $N(x)$ represents a single-output feedforward neural network with network parameters and input datasets (Yadav et al., 2015; Lagaris et al., 1998). A systematic way to demonstrate the construction of the trial solution for treating different common case studies in various scientific fields will be presented in the next section.

Figure 6.2 aims to demonstrate the structure of an ANN with $m$ inputs, a single hidden layer, $h$ nodes in the hidden layer and one linear output. The output of the network, for a given input vector $x := (x_1, \cdots, x_m)$, is given by:

$$N_k = \sum_{j=1}^{h} v_{jk} \sigma_j$$ \hspace{1cm} (6.6)

where

$$\sigma_j = \frac{1}{1 + e^{-a_j}}$$ \hspace{1cm} (6.7)

where

$$a_j = \sum_{i=1}^{m} \omega_{ij} x_i + b_j$$ \hspace{1cm} (6.8)

$\omega_{ij}$ denotes the weight from the input $i = 1, \cdots, m$ to the hidden node $j = 1, \cdots, h$, $v_{jk}$ represents the weight from the hidden node $j$ to the output, $b_j$ is the bias of hidden unit $j$, and $\sigma_j$ stands for the sigmoid transfer function. There are several possibilities of using transfer functions of different types, such as linear, sign, sigmoid and step functions (Yadav et al., 2015). However, the most commonly used function is the sigmoid transfer function (Lagaris et al., 1998).
Figure 6.2: An Artificial Neural Network (ANN) with $m$ inputs, one hidden layer, $h$ nodes in the hidden layer and one linear output.

The $l^{th}$ derivative of the output with respect to the $i^{th}$ input, takes the form:

$$\frac{\partial^l N}{\partial x_i^l} = \sum_{j=1}^{h} v_{jk} \omega_{ij}^{(l)} \sigma_j^{(l)}$$  \hspace{1cm} 6.9

where $\sigma_j^{(l)}$ represents the $l^{th}$ derivative of the sigmoid function.

Once the network structure has been established, and the required conditions have been assumed, the next step will be the minimisation of the objective function. This is achieved by employing almost any optimisation techniques. In this study, nonlinear programming (NLP) optimisation problems were implemented and solved in GAMS using SNOPT and KNITRO as solvers.

It must be noted that in the present work, two-dimensional second-order PDE problems will be treated; however, the methodology can be extended to more dimensions and derivative orders.

**6.2.2 ANN Approximation of the Solution**

Consider the following mathematical model of a PDE problem with Dirichlet boundary conditions (BCs), in which $s = 2$ and $x:=(x_1, x_2)$ where $x \in [x^{LO}, x^{UP}]$. 
Chapter 6

\begin{equation}
J(\partial^2 \Psi, \partial \Psi, \Psi, x) = F_k(\Psi(x), \theta, x) \tag{6.10}
\end{equation}

\begin{align*}
\Psi(x_1^{LO}, x_2) &= F_k^0(x_2) & k \in K \\
\Psi(x_1^{UP}, x_2) &= F_k^1(x_2) & k \in K \\
\Psi(x_1, x_2^{LO}) &= g_k^0(x_1) & k \in K \\
\Psi(x_1, x_2^{UP}) &= g_k^1(x_1) & k \in K
\end{align*}

The ANN network structure can be established for the above single PDE system, resulting in: \( k = 1, l = 2, \) and \( m = 2. \) The two input units of the network are assumed to be: \( x_1 = x \) and \( x_2 = y. \) The form of the trial solution for the PDE model represented by Equation 6.10 is formulated as follows:

\begin{equation}
\Psi_k^{ANN}(x, y) = A(x, y) + x (\lambda_1 - x) \ y (\lambda_2 - y) \ N(x, y) \tag{6.11}
\end{equation}

where an ANN model, \( N(x, y), \) is considered for each trial solution \( \Psi_k^{ANN}(x, y) \). The term \( A(x, y) \) is then formulated as:

\begin{equation}
A(x, y) = (1 - \zeta_1 x) F^0(y) + \zeta_2 x F^1(y) + (1 - \zeta_3 y) [g^0(x) - [(1 - \zeta_1 x) g^0(0) + \zeta_2 x g^0(1)] + \zeta_4 y [g^1(x) - [(1 - \zeta_1 x) g^1(0) + \zeta_2 x g^1(1)]] \tag{6.12}
\end{equation}

Note that \( \Psi^{ANN}(x, y), A(x, y), \lambda_1, \lambda_2, \zeta_1, \zeta_2, \zeta_3 \) and \( \zeta_4 \) satisfy the \textit{Dirichlet} BCs of the PDE model given by Equation 6.10. This therefore facilitates the numerical solution of the PDE model for given values of \( \theta, \) which can be obtained by minimising the error quantity formulated as the following NLP problem (Lagaris et al., 1998):

\begin{equation}
\text{Err}_{PDE} = \min_{\Psi^{ANN}, N, \sigma, \omega, \nu, a, b} \sum_{p \in P} \sum_{k \in K} \left\{ J(\partial^s \Psi_k^{ANN}, \partial^{s-1} \Psi_k^{ANN}, \ldots, \partial \Psi_k^{ANN}, \Psi_k^{ANN}, x^p) - F_k(\Psi(x^p), \theta, x^p) \right\}^2 \tag{6.13}
\end{equation}

If the PDE model given by Equation 6.10 is reformulated with mixed boundary conditions, the neural network approximation of the solution, where \( x_1 = x, x_2 = y, x, y \in [0, 1] \) and \( k = 1, \) is written as (Lagaris et al., 1998):

\begin{equation}
\Psi^{ANN}(x, y) = B(x, y) + x (1 - x) \ y \left[ N(x, y) - N(x, 1) - \frac{\partial N(x, 1)}{\partial y} \right] \tag{6.14}
\end{equation}

Mixed BCs, which involve \textit{Dirichlet} on part of the boundary and \textit{Neumann} elsewhere,
Chapter 6

is of the form:

\[ \Psi(0, y) = \mathcal{F}^0(y) \]  
\[ \Psi(1, y) = \mathcal{F}^1(y) \]  
\[ \Psi(x, 0) = \mathcal{g}^0(x) \]  
\[ (\partial \Psi(x, 1) / \partial y) = \mathcal{g}^1(x) \]

The term \( B(x, y) \), of the trial solution (Equation 6.14) is chosen to satisfy the mixed BCs (Lagaris et al., 1998):

\[ B(x, y) = (1 - x)\mathcal{F}^0(y) + x\mathcal{F}^1(y) + \mathcal{g}^0(x) \]

\[ -[(1 - x)\mathcal{g}^0(0) + x\mathcal{g}^0(1)] \]

\[ + y \{ \mathcal{g}^1(x) - [(1 - x)\mathcal{g}^1(0) + x\mathcal{g}^1(1)] \} \]

The trial solutions presented above allow us to treat PDE models with orthogonal box boundaries. It however poses a challenge when the aim is to deal with realistic problems whose the boundaries are highly irregular. One of the key contributions of this thesis is to develop a meshless methodology for parameter estimation, capable of dealing with any arbitrarily complex geometrical shape. This is achieved by choosing a trial solution in such a way so as to satisfy the differential equation. More specifically, the boundary conditions can be exactly satisfied by picking points on the boundary and hence the network is trained to satisfy the differential equation. The model suitable for this case can be written as:

\[ \Psi_k^{ANN}(x, y) = N_k(x, y) \]

Different numerical example problems which demonstrate the capabilities of the proposed approach will be presented in the next section. According to the numerical experiments, the ANN-based methodology based upon the formulation presented in this section has been proven to be very effective by providing accurate solutions in reasonable computing times. Moreover, the reported solution accuracy can be improved further by calibration of nodes within the ANN hidden layer in order to compute the optimal ANN topology.

Before proceeding with the numerical analysis, it is worth noticing that the generic mathematical formulation of the parameter estimation problem involves minimisation
of the LS objective function, Equation 6.2, subject to the PDE model, Equation 6.3, and associated BCs, and the ANN model, Equations 6.4-6.9.

6.3 Numerical Validation of the Model Accuracy

In this section, a number of case studies will be presented to demonstrate the advantages of the proposed modelling framework for the parameter estimation of partial differential equations. To computationally test and illustrate the performance of the proposed methodology for estimating unknown parameters in PDE models, the following example problems will be treated. The first problem seeks to estimate the diffusivity in the heat equation; the second one considers a linear Poisson equation with Dirichlet boundary conditions (BCs) while the third one studies the linear Poisson equation with mixed BCs; the fourth example problem examines a non-linear Poisson equation with mixed BCs; and the last one treats a highly non-linear problem with an irregular boundary. In all models with orthogonal box boundaries, the domain was taken to be $[0,1] \times [0,1]$ considering both uniform and non-uniform grid discretisation. A summary of the problems and the solutions obtained is given in Table 6.1 in Section 6.4.

All the optimisation problems were formulated as NLPs and solved using GAMS 24.7.1 (Rosenthal, 2008) on a Dell workstation with 3.00 GHz processor, 8GB RAM, and Windows 7 64-bit operating system. It should be noted that the main difficulty with the parameter estimation arises from the non-convexity of the non-linear objective function, as minimisation of such functions may result in different local optimal solutions. For this reason, the parameter estimation results may change for various NLP solvers and initial parameter guess values used for the solvers. Each solver can handle certain model types, therefore the objective in this work is to choose an appropriate solver allowing for optimal solutions to be computed in reasonable CPU times. To this end, the optimisation problems corresponding to the PDE models with orthogonal box boundaries were modelled in GAMS 24.7.1 and solved using SNOPT, while those corresponding to the PDE models with irregular boundaries were solved using KNITRO.

6.3.1 Problem 1

A numerical example is presented for the estimation of the diffusivity in the heat equation with Dirichlet BCs. The model is a linear PDE of parabolic type in one
dimension of time and one space dimension.

### 6.3.1.1 Parameter Estimation using Uniform Grid

Consider the following partial differential equation with associated boundary and initial conditions, representing a mathematical model for a system governed by the heat equation (Seinfeld and Chen, 1971):

\[
\theta \frac{\partial^2 \psi}{\partial x^2} = \frac{\partial \psi}{\partial t} \tag{6.18}
\]

\[
\psi(0, x) = \sin \pi x \quad 0 \leq x \leq 1
\]

\[
\psi(1, x) = 0
\]

\[
\psi(t, 0) = \psi(t, 1) = 0 \quad 0 \leq t \leq 1
\]

in which \( \psi = \psi(t, x) \) denotes the state variable representing the temperature profile, \( x \) is the space coordinate, \( t \) is the time, and the model parameter \( \theta \in \mathbb{R}^{n_\theta}; n_\theta \in \mathbb{N} \), stands for the thermal diffusivity which is unknown throughout the parameter estimation problem.

For this example problem, PSE’s gPROMS® advanced process modelling platform was used for the generation of the simulated measurement data. The PDE model (Equation 6.18) was numerically solved by setting the actual value of the unknown parameter as \( \theta = 1 \). The model was implemented in gPROMS while the partial differential equation describing the heat transfer process was simulated using OCFE scheme. To obtain a precise numerical solution, both time and space domains were to be handled using third order orthogonal collocation over ten finite elements.

Having simulated measurement data, the parameter estimation problem was formulated and solved in GAMS using ANN model. Note that to approximately solve the heat equation using an ANN, the trial form of the solution must be written as:

\[
\psi^{ANN}(t, x) = (1 - t) \sin \pi x + t (1 - t) x (1 - x) N(t, x) \tag{6.19}
\]

As discussed earlier in Section 6.2.1, the trial solution (Equation 6.19) should be chosen such that the initial/boundary conditions of the PDE model are satisfied. Therefore, by incorporating the four boundary points given in Equation 6.18, into
Chapter 6

Equation 6.11, \( \lambda_1 = \lambda_2 = 1 \) is obtained, while \( A(t, x) = (1 - t) \sin \pi x \) is found by direct substitution in the general form given by Equation 6.12. Considering a uniform square discretisation of the domain \([0, 1] \times [0, 1]\), solving the parameter estimation problem gives \( Err_{PE} = 6.3643 \times 10^{-6} \) and \( \theta = 0.98863 \) as the parameter estimate.

6.3.1.2 Parameter Estimation using Non-Uniform Grid

To show the ability of the ANN-based simultaneous formulation for estimating unknown parameters, a non-uniform grid discretisation is now investigated in this section. A desirable feature of the ANN-based approach is that random points of each variable can be chosen over the domain resulting in a non-uniform grid. This could be useful in PDE models with irregular boundaries in which more sample points might be required in some regions of the domain.

![Figure 6.3: Accuracy of the computed solutions corresponding to problem 1 at the training points. The parameter estimation problem was formulated and solved in GAMS using uniform grid (navy points) and non-uniform grid (red points). The ANN-based model predictions are validated by comparisons with simulation carried out in gPROMS (blue surface).](image)

The network architecture is now considered to be an ANN with two inputs \( x^P := (x^P, t^P) \), one hidden layer and twenty nodes in the hidden layer. For performing
training, a total of 121 data points, \( p := (1, 2, \cdots, 11) \), are obtained by considering nine random points of the domain \((0, 1)\) of each variable and four boundary points as: \( x^1 = 0, x^{11} = 1, t^1 = 0 \) and \( t^{11} = 1 \). Solving the parameter estimation problem for this case study gives \( \text{Err}_{PE} = 1.82757 \) and \( \theta = 0.99603 \) as the parameter estimate. Computational times for the obtained results are approximately 40.5 seconds for the uniform grid and 226.8 seconds for the non-uniform grid. A comparison of the model predictions between the ANN-based formulation in GAMS and the method of OCFE in gPROMS is given in Figure 6.3. It is observed that there is a good agreement in the model predictions across both modelling platforms.

### 6.3.2 Problem 2

Consider the following Poisson equation with Dirichlet BCs, which is a partial differential equation of elliptic type (Lagaris et al., 1998):

\[
\nabla^2 \Psi(x, y) = e^{-x}(x - \theta_1 + y^3 + \theta_2 y)
\]

6.20

\[
\Psi(0, y) = y^3
\]

\[
\Psi(1, y) = (1 + y^3) e^{-1}
\]

\[
\Psi(x, 0) = xe^{-x}
\]

\[
\Psi(x, 1) = e^{-x}(x + 1)
\]

where the actual values of the parameters are \( \theta = [\theta_1 \theta_2] = [2 6] \), and \( x, y \in [0,1] \). The analytical solution for the above PDE model is as follows:

\[
\Psi_{\text{analytic}}(x, y) = e^{-x}(x + y^3)
\]

6.21

To illustrate the performance of the proposed methodology, the vector of parameters in Equation 6.20 is assumed to be unknown and must be estimated by formulating and solving the parameter estimation problem. The domain \([0,1] \times [0,1] \) was taken with a uniform grid discretisation considering a mesh of 36 points obtained by subdivideing the interval in five equal subintervals corresponding to six equidistant points in each direction. Using Equation 6.11, the trial solution of the PDE model must be written as \( \Psi^\text{ANN}(x, y) = A(x, y) + x(1 - x)y(1 - y)N(x, y) \). The term, \( A(x, y) \), can be obtained by direct substitution in the general form given by Equation 6.12:
Chapter 6

\[ A(x, y) = (1 - x) y^3 + x (1 + y^3) e^{-1} + (1 - y) x (e^{-x} - e^{-1}) \]

\[ + y [(1 + x)e^{-x} - (1 - x + 2 x e^{-1})] \]

Equation 6.22 incorporates the BCs given in Equation 6.20. Parameter estimation problem was modelled and solved in GAMS. As indicated in Figure 6.4, the ANN-based solution is comparable to the analytical solution. Solving the parameter estimation problem for the uniform grid discretisation provides \( \text{Err}_{FE} = 2.7615 \times 10^{-6} \) and \( \theta = [\theta_1, \theta_2] = [2.03029, 6.00006] \), and required only 8.6 seconds of computation time. The computational experiment was carried out for ten nodes in the hidden layer.

**Figure 6.4:** Accuracy of the computed solutions corresponding to problem 2 at the training points by comparing the model predictions against the analytical solution (blue surface). The parameter estimation problem was formulated and solved in GAMS using uniform grid (navy points) and non-uniform grid (red points).

It is interesting to explore the advantage of ANN-based framework for estimating the model parameters over a non-uniform grid, when a small number of points is available for performing training. A non-uniform grid was generated by considering four random points of the domain \((0, 1)\) of each variable and four boundary points as the following: \( x^1 = 0, x^6 = 1, y^1 = 0 \) and \( y^6 = 1 \). Using 7 nodes in the hidden layer,
\[ \theta = [\theta_1 \quad \theta_2] = [2.00926 \quad 5.99466], \] and an error of \( \text{Err}_{PE} = 1.919 \times 10^{-4} \) were obtained, and it took approximately 22 seconds to converge. The solution accuracy is shown in Figure 6.4 demonstrating that the random training points (red points) are superimposed on the exact solution of the PDE problem.

### 6.3.3 Problem 3

Let us consider a PDE model representing a Linear Poisson Equation with mixed BCs as stated as follows (Lagaris et al., 1998):

\[
\nabla^2 \Psi(x, y) = (2 - \theta^2 y^2) \sin(\pi x) \tag{6.23}
\]

\[
\Psi(0, y) = 0
\]

\[
\Psi(1, y) = 0
\]

\[
\Psi(x, 0) = 0
\]

\[
(\partial \Psi(x, 1) / \partial y) = 2 \sin(\pi x)
\]

where the actual value of the parameter is \( \theta = \pi \), and \( x, y \in [0, 1] \). As before, a uniform grid discretisation is first studied; hence, training was performed using a mesh of 121 points obtained by considering eleven equidistant points of the domain \([0, 1]\) of each variable. For constructing the ANN topology, one hidden layer with ten hidden nodes were used for this case study.

The analytical solution of the given PDE model (Equation 6.23) is stated as follows, and displayed in Figure 6.5.

\[
\Psi_{\text{analytic}}(x, y) = y^2 \sin(\pi x) \tag{6.24}
\]

Using Equation 6.14, the trial solution of the PDE model must be written as

\[
\Psi^{\text{ANN}}(x, y) = B(x, y) + x (1 - x) y \left[ N(x, y) - N(x, 1) - \frac{\partial N(x, 1)}{\partial y} \right].
\]

The term, \( B(x, y) \), can be achieved by direct substitution in the general form given by Equation 6.16:

\[
B(x, y) = 2 \ y \sin(\pi x) \tag{6.25}
\]

Results concerning the accuracy of the approximate solution obtained by using the suggested methodology are presented in Figure 6.5. It is clear that the ANN-based
solution is in a good agreement with the exact solution. Solving the parameter estimation problem provides an error of $\text{Err}_{PE} = 0.01657$ in about 258.8 seconds, and the computed parameter estimate is $\theta = 3.14123$. For a non-uniform grid of nine random points in $(0,1)$, $\text{Err}_{PE} = 0.01167$ and $\theta = 3.14325$ were obtained for ten nodes in the hidden layer and it took about 84 seconds for the convergence of the algorithm.

![Figure 6.5: Accuracy of the computed solutions corresponding to problem 3 at the training points by comparing the model predictions against the analytical solution (blue surface). The parameter estimation problem was formulated and solved in GAMS using uniform grid (navy points) and non-uniform grid (red points).](image)

### 6.3.4 Problem 4

A nonlinear PDE problem (Lagaris et al., 1998) with the same mixed BCs as in Problem 3, is treated in this section. The analytical solution and the neural network approximation of the solution are the same with those of Problem 3. However, the mathematical model is given by:

$$\nabla^2 \Psi(x,y) + \Psi(x,y) \frac{\partial}{\partial y} \Psi(x,y) = \sin(\pi x) \left( 2 - \theta_1^2 y^2 + \theta_2 y^3 \sin(\pi x) \right)$$

where the actual values of the parameters are $\theta = [\theta_1 \ \theta_2] = [\pi \ 2]$, and $x, y \in [0,1]$. 

140
The network was first trained using a uniform grid of six equidistant points in [0, 1]. Parameter estimation problem was solved for twelve hidden nodes for a uniform grid to give $\text{Err}_{PE} = 4 \times 10^{-5}$ and $\theta = [3.11367, 1.97794]$. By considering seventeen nodes in the hidden layer for a non-uniform grid, $\text{Err}_{PE} = 1 \times 10^{-10}$ and $\theta = [3.22134, 1.97967]$ were obtained. In Figure 6.6, a very close match is obtained between the analytical solution and the model predictions. Convergence was achieved in 492 and 68 CPU seconds for uniform and non-uniform grid, respectively.

![Figure 6.6: Accuracy of the computed solutions corresponding to problem 4 at the training points by comparing the model predictions against the analytical solution (blue surface). The parameter estimation problem was formulated and solved in GAMS using uniform grid (navy points) and non-uniform grid (red points).](image)

### 6.3.5 Problem 5

Consider the following highly nonlinear problem (Lagaris et al., 2000) with a star-shaped domain as shown in Figure 6.7.

$$\nabla^2 \psi(x, y) + e^{\psi(x,y)} = 1 + x^2 + y^2 + \frac{4}{(\theta + x^2 + y^2)^2}$$

where the actual value of the model parameter is $\theta = 1$ and $x, y \in [-1,1]$. The star-
shaped boundary has twelve vertices and sides. The boundary points \((x, y)\) on the definition domain are considered by picking points on the interval \([-1, 1]\) on the \(x\) axis and \(y\) axis, respectively. The total number of points taken on the boundary is 60, and a total of 171 points were taken within the star-shaped domain. Using the analytical solution, \(\Psi_{\text{analytic}}(x, y) = \log(1 + x^2 + y^2)\), the values of the state variable at the boundary points were computed and have been used in the training.

Figure 6.7: The star-shaped domain (171 points) and the boundary points (60 points) corresponding to Problem 5.

The unknown model parameter can be estimated while simultaneously computing the model predictions for the state variable. Solving the parameter estimation problem for the above PDE model yields \(\text{Err}_{PE} = 1.2749 \times 10^{-4}\) and \(\theta = 1.57098\), and required 158.48 seconds of computation time. The accuracy of the obtained solution using an ANN with nineteen hidden nodes is presented in Figure 6.8. The proposed approach for parameter estimation works well for PDE models with arbitrarily complex boundaries. As indicated here, a close estimate of the parameter is made and the approximate solution is of high accuracy since there is a good match between the exact solution and the model predictions.
6.4 Conclusion

The aim of this chapter was to propose a new approach for parameter estimation of a system of PDEs. A number of factors must be considered in the development of a computationally efficient parameter estimation scheme for PDE models (Polis et al., 1973; Seinfeld and Chen, 1971):

- No restrictions on the form of the differential equations and the boundary geometry;
- No restrictions on the measured data, and the number of spatial locations and temporal points at which data is taken;
- Saving in computational times;
- Obtaining highly accurate parameter estimates and model predictions.

To address the above factors, a parameter estimation framework based on the
neural network (ANN) approximations was developed and tested extensively on different example problems. To evaluate the performance of the suggested methodology, five numerical examples were experimented with a mesh-grid of small and moderate size, considering different distributions (uniform and non-uniform) with boundary conditions (Dirichlet and Neumann) defined on boundaries with simple and complex geometry. A summary of the results obtained from solving the parameter estimation problem using the ANN scheme is presented in Table 6.1.

Table 6.1: Example problems 1 – 5.

<table>
<thead>
<tr>
<th>Problem</th>
<th>Grid discretisation</th>
<th>Parameter</th>
<th>Actual value</th>
<th>Estimate</th>
<th>Error (ErrPE)</th>
<th>CPU time (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Problem 1</td>
<td>Uniform</td>
<td>$\theta$</td>
<td>1</td>
<td>0.98863</td>
<td>$6.3643 \times 10^{-6}$</td>
<td>40.5</td>
</tr>
<tr>
<td></td>
<td>Non-uniform</td>
<td>$\theta$</td>
<td>1</td>
<td>0.99603</td>
<td>1.82757</td>
<td>226.8</td>
</tr>
<tr>
<td>Problem 2</td>
<td>Uniform</td>
<td>$\theta_1$</td>
<td>2</td>
<td>2.03029</td>
<td>$2.7615 \times 10^{-6}$</td>
<td>8.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\theta_2$</td>
<td>6</td>
<td>6.00006</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non-uniform</td>
<td>$\theta_1$</td>
<td>2</td>
<td>2.00926</td>
<td>$1.919 \times 10^{-4}$</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\theta_2$</td>
<td>6</td>
<td>5.99466</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Problem 3</td>
<td>Uniform</td>
<td>$\theta$</td>
<td>$\pi$</td>
<td>3.14123</td>
<td>0.01657</td>
<td>258.8</td>
</tr>
<tr>
<td></td>
<td>Non-uniform</td>
<td>$\theta$</td>
<td>$\pi$</td>
<td>3.14325</td>
<td>0.01167</td>
<td>84</td>
</tr>
<tr>
<td>Problem 4</td>
<td>Uniform</td>
<td>$\theta_1$</td>
<td>$\pi$</td>
<td>3.11367</td>
<td>$4 \times 10^{-5}$</td>
<td>492</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\theta_2$</td>
<td>2</td>
<td>1.97794</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non-uniform</td>
<td>$\theta_1$</td>
<td>$\pi$</td>
<td>3.22134</td>
<td>$1 \times 10^{-10}$</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$\theta_2$</td>
<td>2</td>
<td>1.97967</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Problem 5</td>
<td>Non-uniform</td>
<td>$\theta$</td>
<td>1</td>
<td>1.57098</td>
<td>$1.2749 \times 10^{-4}$</td>
<td>158.48</td>
</tr>
</tbody>
</table>

Varying the ANN topology will have different computational demands such as the prediction accuracy and the central processing unit (CPU) times for estimating parameters. A trade-off between the solution accuracy and the computational time is required to land on an optimal configuration of the ANN model. The highest prediction accuracy with minimum computational time was achieved using a single hidden layer ANN model. The computational demands required to converge to the optimal solution are presented in Table 6.1. The illustrative examples provided in this chapter demonstrate that the ANN-based approach is very efficient by providing accurate solutions in reasonable computing times. It must be noted that the proposed approach can be extended to deal with a system of PDEs where the required number
of parameters becomes large. Thus, the optimisation problem may require more computational time and memory. The proposed methodology can be used for modelling gene delivery systems involving temporal and spatial variations of genetic materials within the cell. The developed approach in this work makes it feasible to solve more complex gene delivery models on much more complex domains, which should be the focus of future research.
Chapter 7.

7 CONCLUSIONS AND FUTURE WORK

7.1 Summary of Thesis and Key Contributions
The main purpose of this work has been to demonstrate the mathematical modelling and model-based control strategies for gene delivery systems. This thesis extends the state-of-the-art modelling techniques by developing an innovative approach based on the optimal control strategy in order to optimise the process of gene delivery. More broadly, by tackling the ambitious challenge of gene therapies, this work provided an effective decision-making platform that can serve as a starting point in allowing for feasible control of the gene transfer systems, paving the path for further advancements in the next-generation delivery device systems capable of capturing the complexity of in vivo conditions.

The main contributions of this work are as follows:
Chapter 7

7.1.1 Optimal Model-Based Control of Non-Viral siRNA Delivery

One of the key achievements of this PhD thesis, which was presented in Chapter 4, was the development of an integrated PK/PD model-based control algorithm for non-viral siRNA delivery to take into account the main multi-objective optimisation issues, such as efficacy and toxicity, while considering the effect of variations in cell division time on transfection. The methodology developed in this study provides an effective model-based tool for making decisions under uncertainty, which is lacking for gene delivery systems. The proposed modelling and control platform allows for the computation of an optimal infusion profile in the presence of different practical constraints, making it applicable for in vivo conditions. A constrained multi-objective optimisation problem was formulated and solved to achieve maximum gene silencing activity while simultaneously preserving cell viability at desirable levels. Effect of variations in cell doubling time was also explored during the modelling process by incorporating time constraints into the optimisation problem to improve the pharmacological effects before cell division occurs. The results obtained from the work show that an optimal continuous infusion is superior to a bolus injection for achieving maximum therapeutic effects with minimal toxicity. The analysis successfully provides quantitative predictions of non-viral siRNA activity paving the path for further experimental work to probe more efficient delivery systems.

7.1.2 Mathematical Modelling of Gene Delivery in Haemophilia Patients

The primary motivation of the work presented in Chapter 5, was the development of a modelling framework to help predict the outcome of a given dose of vector in patients with haemophilia B, which could be used to aid clinical decisions. However, the predictive models incorporate parameters that must be estimated before the models can be used for prediction. Therefore, the proposed modelling approaches were classified into two groups: (i) the individual modelling approach, where the PK/PD parameters were characterised to individual patients, and (ii) the population modelling approach in which the model parameters were estimated simultaneously for all patients. Although the results obtained from both modelling frameworks were shown to agree well with the reported clinical data, a better model prediction was achieved from the individual modelling approach suggesting that a personalised treatment may be more suitable. As the model becomes more personalised, the
prediction becomes more accurate. However, the initial dose selection was demonstrated through a simulation study based upon the population modelling approach, which consequently forms a general problem formulation. This is particularly important for new patients who enter the treatment. The initial vector dose could be determined based on the population model, and the algorithm can be used to predict the pharmacokinetics and pharmacodynamics of the vector during the therapy.

7.1.3 Parameter Estimation of Partial Differential Equations using Artificial Neural Network

In order to develop a high-fidelity spatial-temporal mathematical model for gene delivery systems, an efficient and reliable approach for parameter estimation is required to account for domains with irregular boundaries so as to be capable of dealing with any arbitrarily complex geometrical shape. In Chapter 6, a new methodology based on the ANN formulation was presented for estimating parameters in systems described by partial differential equations. This approach is able to deal with linear and nonlinear PDEs, with Dirichlet and Neumann boundary conditions, considering both regular and irregular boundaries. One of the main advantages of this method is that the ANN-based formulation offers a meshless framework to consider arbitrarily complex boundaries. A simultaneous solution and optimisation strategy was presented via ANNs to account for non-uniform arbitrary regions which widely exist in real-world phenomena such as complex cellular architectures. The applicability of the proposed methodology was demonstrated with different numerical problems. The ANN-based approach was shown to be very efficient by providing accurate solutions in reasonable computing times.

7.2 Future Work

The research presented in this work opened up a variety of research directions that could be pursued in the future. This section will focus on potential steps for future research developments.

7.2.1 Control-Relevant Modelling in Gene Delivery

The model-based control strategy proposed in this study has paved the way to inspire development of technology in new discoveries of computer-controlled infusion pumps in order to improve the pharmacological effects of the therapeutic genes.
While the pioneering results in this study confirm the potential of control algorithms in optimizing the gene delivery systems, the hurdles to put it into practice remain to be overcome. The transition to the practical arena may reveal unforeseen challenges, mainly in the transfer of a precise (optimal) amount of therapeutic genes that poses a key limitation in the application of this technology. The model-based controller algorithm proposed in this work, requires a computer-controlled infusion pump, so that the system would calculate the exact amount of optimal infusion rate designed to maintain the pharmacological effects at desirable levels, and a signal would drive an infusion pump to deliver the exact optimal amount of therapeutic agents. While infusion pumps for delivery of drugs can be realized, development of infusion pumps that can deliver precise amounts of the genetic material remains to be seen. It is then envisaged that this work will trigger enthusiasm for development of such pumps. Therefore, the development of a computer-controlled gene delivery device system should be the focus of future research. A potential direction for the future work is to design an integrated infusion procedure composed of a control system involving a computing platform and a sensor to be connected to a programmable infusion pump along with an appropriate physical delivery method, such as a syringe pump (Sanftner et al., 2005) or a gene gun (Iida et al., 1990), which could provide and deliver the desired amount of therapeutic agents into a patient’s body in a controlled manner.

### 7.2.2 Computational Modelling of Reaction-Diffusion Processes in Gene Delivery

As future directions of work, one may extend the mathematical model by including the reaction-diffusion mechanisms to afford a more realistic representation of cellular processes in gene delivery. The system will involve spatially distributed compartments to consider the cellular geometry, which will be modelled using partial differential equations (PDEs). The parameter estimation framework based on the neural network approximations, which was developed in Chapter 6, has paved a way into meshless modelling of simple and complex gene delivery systems, allowing the diffusion to be considered in the models. The extra complexity in models is not well supported by the available data, thus in order to be able to develop such a mathematical framework, experimental measurements of spatiotemporal distribution of gene carriers are required.
7.2.3 Model-Based Optimal Control of Gene Delivery in Patients with Haemophilia B

In Chapter 5, mathematical models were developed based on the available clinical data for three patients with severe haemophilia B. A number of future directions can be investigated in this area of study, which are stated as follows:

(a) Future work will include the development of model-based optimal control algorithm to compute an optimal infusion profile aiming at maximising the total plasma factor IX activity, while minimising the liver toxicity level (ALT level). Having the PK/PD model, the gene delivery optimal control problem can then be posed as the following constrained optimisation problem:

\[
\max_{q(t)} \int_{t=0}^{t=t_f} R_{\text{FIX}}(t) \, dt \equiv \Delta t \sum_{t=0}^{t=t_f} R_{\text{FIX}}(t)
\]

subject to: the PK/PD models, initial conditions, and

\[
R_{\text{ALT}}(t) \leq R_{\text{ALT}}^{UP}
\]

where \(R_{\text{FIX}}\) denotes the plasma factor IX coagulation activity level, \(R_{\text{ALT}}^{UP}\) represents the maximum acceptable level of liver toxicity, and \(t_f\) is the final time at the end of the therapy. To address the uncertainty, the optimal control problem can be solved for different values of \(R_{\text{ALT}}^{UP}\).

(b) In the clinical study conducted by Nathwani et al. (2014), prophylactic factor IX concentrate was used after gene transfer for preventing spontaneous bleeding episodes, which has influenced the plasma FIX activity levels. Thus, another potential direction for this work is the incorporation of prophylaxis with factor IX concentrate into the developed models. The aim is to investigate the effect of these treatments on the modelling of gene delivery system.

(c) The current modelling approach considers a lumped representation of the vector. Future work should focus on a detailed PK/PD model to incorporate the liver compartment, indicating a more realistic representation of the body. To this purpose, the liver should be biopsied after gene transfer for direct measurements.
References


Bibliography


Bibliography

Microbiology Reviews, 11(1), 42-56.


Bibliography


Bibliography


Bibliography


Bibliography

Reviews Genetics, 12(5), 316-328.


of America, 102(21), 7523-7528.


Bibliography


Bibliography

viral vectors expressing the human factor IX cDNA. Blood, 97(5), 1258-1265.


Neuman, C. P. & Sen, A. (1973) Suboptimal control algorithm for constrained


Bibliography

Publishers Inc.


Bibliography

Gene Therapy, 12(13), 1023-1032.


