# TABLE OF CONTENTS

Acknowledgements .................................................. i  
Tables of Contents ............................................... iii 
Abbreviations ....................................................... vi 

## CHAPTER 1  INTRODUCTION 
1.1 Background .................................................. 1 
1.2 Initial Infection and Host Response ......................... 2 
1.3 Drug Delivery in Tuberculosis ................................ 4 
1.4 Objective ..................................................... 9 

## CHAPTER 2  MATERIALS AND METHODS 
2.1 Preparation of Microparticles  
2.1.1 Materials .................................................. 12 
2.1.2 Multiple Emulsion Solvent Evaporation Technique .... 12 
2.1.3 Spray Drying ............................................... 14 
2.1.4 MP Characterization ....................................... 16 
2.2 Analytical Method Development and Validation  
2.2.1 Materials .................................................. 17 
2.2.2 HPLC Instrumentation and Chromatographic Conditions .................................................. 17 
2.2.2.1 Method Development and Validation in "inert matrix" .................................................. 18 
2.2.2.2 Method Development and Validation in "Biomatrix" .................................................. 21 
2.2.3 LCMS Method Development .................................. 27 
2.3 Delivery of Inhalable Microparticles to Rodents  
2.3.1 Characterization of Biodegradable Microparticles ...... 29 
2.3.1.1 Particle Size Distribution by Laser Scattering ........... 29 
2.3.1.2 Aerodynamic Particle Sizing by Inertial Impaction .......... 29 
2.3.1.3 In-vitro release studies .................................. 31 

Targeted Delivery of Inhalable Microparticles for Pulmonary Tuberculosis  

Table of Contents
Table of Contents

2.3.2 Design and Standardization of Inhalation Apparatus ..... 31
  2.3.2.1 Experimental .................................. 32
2.3.3 In Vivo delivery of Microparticles ....................... 32
2.3.4 Single Dose Bioavailability ................................ 36

2.4 Microparticle Interaction with Target Cells
  2.4.1 Isolation of Macrophages ................................ 37
    2.4.1.1 Heterogeneity of cell population obtained by BAL 37
    2.4.1.2 Percoll Density Gradient Separation ............. 38
    2.4.1.3 Magnetic Bead Separation ....................... 39
  2.4.2 Co-Localization of MP with Infected Macrophages In-Vitro 39
  2.4.3 Extra-Cellular Cytokine Profile in Infected and Treated Cells ........................................ 40
  2.4.4 Intra-Cellular Cytokine Estimation by Flow Cytometry ........................................ 42

CHAPTER 3 RESULTS AND DISCUSSION

3.1 Preparation of Microparticles
  3.1.1 Multiple Emulsion Solvent Evaporation Technique ... 45
  3.1.2 Spray Drying ........................................ 46

3.2 Analytical Method Development and Validation
  3.2.1 Method Development and Validation in "inert matrix" 51
  3.2.2 Method Development and Validation in "Biomatrix".. 55
  3.2.3 LCMS Method Development .............................. 61

3.3 Inhalable Microparticles and their Delivery to Laboratory Animals
  3.3.1 Characterization of Biodegradable Microparticles 65
    3.3.1.1 Particle Size Distribution by Laser Scattering 66
    3.3.1.2 Aerodynamic Particle Sizing by Inertial Impaction ....................... 67
    3.3.1.3 In-vitro release studies .......................... 69
  3.3.2 Design and Standardization of Inhalation Apparatus .... 71
Table of Contents

3.3.2.1 Experimental Design ................................. 73
3.3.3 Determination of Inhaled Dose ............................. 75
3.3.4 Impact of macrophage yield on dose determination 78
3.3.5 Drug distribution in sampled compartments 79

3.4 Microparticle Interaction with Target Cells

3.4.1 Isolation of Macrophages ...................................... 80
  3.4.1.1 Heterogeneity of cell population obtained by BAL 82
  3.4.1.2 Density Gradient Fractionation of Lungs ............ 83
  3.4.1.3 Magnetic Bead Separation ............................ 84

3.4.2 Co-Localization of MP with Infected Macrophages
  In-Vitro ..................................................... 84

3.4.3 Extra-Cellular Cytokine Profile ............................ 85
3.4.4 Intra-Cellular Cytokine Profile ............................ 87

SUMMARY & CONCLUSIONS ............................................. 89

REFERENCES ................................................................. 90

APPENDIX ................................................................. 103