DEVELOPMENT OF AN ELUTING-SUPRAMOLECULAR ASSEMBLED CONTACT LENS-LIKE DEVICE FOR THE TOPICAL BIOGENIC TREATMENT OF CATARACTS

Zikhona Hayiyana

A dissertation submitted to the Faculty of Health Sciences, University of the Witwatersrand, in fulfillment of the requirements for the degree of Master of Pharmacy

Supervisor:

Professor Viness Pillay

University of the Witwatersrand

Department of Pharmacy and Pharmacology

Wits Advanced Drug Delivery Platform Research Unit

South Africa

Co-supervisors:

Professor Yahya E. Choonara University of the Witwatersrand,

Department of Pharmacy and Pharmacology, South Africa

Professor Lisa C. du Toit University of the Witwatersrand,

Department of Pharmacy and Pharmacology, South Africa

Mr. Pradeep Kumar University of the Witwatersrand,

Department of Pharmacy and Pharmacology, South Africa

Johannesburg, 2016

DECLARATION

I, Zikhona Hayiyana, declare that this dissertation is my own work. It is being submitted for the degree of Master of Pharmacy in the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg. It has not submitted before for any degree or examination at this or any other University.

…….

Signed at ……..on this…. day of November 2016

ACKNOWLEDGEMENTS

I would like to sincerely thank and appreciate my supervisor, Professor Viness Pillay for allowing me the opportunity to advance my education under his supervision and for providing all the necessary resources to ensure the completion of this project. I would also like to thank my co-supervisors Professor Yahya Choonara, Professor Lisa du Toit and Mr. Pradeep Kumar, your hard work and continuing assistance has brought this project to fruition.

My sincerest appreciation goes to the Department of Health of Mpumalanga for funding my undergraduate studies, the NRF, GDARD and Wits University for providing funds that made to it possible that this project is completed.

Thank you to my siblings (Siyavuya, Ndimzukise, Nondzukiso, Yomelela, Dunyiswa and Hlalunam) for being my strength and inspiration. To my father and mother, the late Mbuso Hayiyana and Nomanesi Mkhangeli, Thank you for giving me life and raising me to believe in myself. To Mr Sintu Guma and Mrs Phumeza Guma thank you because all this was only possible because you took a step of faith and believed in me.

Thank you to Mr. Sello Ramarumo, the Central Animal Services (CAS) staff, the lab technicians and fellow colleagues for all the support provided to ensure the completion of this project.

Above all, to God is the Glory, now and forever more, Amen.

DEDICATION

I would like to dedicate this work to my siblings. I hope that it will remind you that we truly can achieve all things and no mountain is too high for us to climb. Keep pushing. All things are possible to those who believe. Let us therefore continue to climb to higher dimensions.

RESEARCH OUTPUTS

1. Review Paper

a. An overview of cyclodextrins and multi-cyclodextrin supramolecular entities for advanced drug delivery applications. To be submitted to the Pharmaceutical Development and Technology Journal.

2. Research Papers

a. Hayiyana Z., Choonara Y.E., Makgotloe M.A., du Toit L.C., Kumar P., Pillay V. Ester-based hydrophilic cyclodextrin nanosponges for topical ocular drug delivery. Current Pharmaceutical Design. ***Accepted for publication***, May 2016. (Appendix A1).

b. A nanosponge-modified stimuli sensitive sol-gel system for ocular drug delivery: For submission to an ISI-accredited international journal (Journal of Inclusion Phenomena and Macrocyclic Chemistry).

c. *In vivo* therapeutic assessment of a bioactive supramolecular-assembled *in situ* gelling system in cataract induced in rabbits: For submission to an ISI-accredited international journal (Experimental Eye Research Journal).

CONFERENCE PRESENTATIONS

1. Podium

a. Hayiyana Z., du Toit L.C., Choonara Y.E., Kumar P., Pillay V. A nanosponge-modified stimuli sensitive *in situ* gelling system for ocular drug delivery (Podium Presentation). Academy for Pharmaceutical Sciences of South Africa conference, Sandton Convention Centre, Johannesburg, South Africa, 2015. (Appendix B1).

2. Poster

Hayiyana Z., du Toit L.C., Choonara Y.E., Kumar P., Pillay V. Design and development of a bioactive nanosponge-modified *in situ* gelling system (Poster Presentation). Cross-faculty PG Symposium, University of the Witwatersrand, Johannesburg, South Africa, 2016. (Appendix B2).

ANIMAL ETHICS CLEARANCE

I hereby confirm that the study entitled: '*In vivo* assessment of topical ocular drug delivery systems in rabbits', received approval from the Animal Ethics Screening Committee of the University of the Witwatersrand. Ethics clearance number: 2014/66/C (See Appendix C1).

PATENT APPLICATION

A Provisional Patent will be submitted to the South African Provisional Patents Association.

ABSTRACT

This project tackles two main issues: poor ophthalmic drug bioavailability and treatment alternatives for cataract. A synergistic approach of developing a bi-composite drug delivery system with components possessing the capacity to improve drug solubility, drug stability, residence time, amount of drug available at the membrane surface and membrane permeability was developed through the application of highly potent nanosponges and an *in situ* gelling system.

The newly synthesized bi-composite system was assessed for toxicity using the Epiocular Tissue Model™ and was found to be non-toxic with a Draize score of 18.15. It was then assessed for potential to deliver bioactives *in vivo* using 2-2.5kg New Zealand White Rabbits. Two main administration routes were tested; the topical and intravitreal route. Assays for detection of the bioactives from the aqueous humor, lens and vitreous humor were performed and quantitative analysis was performed using Ultra Performance Chromatography (UPLC). The bioactive levels reached up to 80% saturation in some ocular tissue sites. The next step involved a second *in vivo* study where cataract was induced on 4-5 weeks New Zealand White pups using diquat dibromide. Following confirmation of cataract development by slit lamp illumination, the bi-composite drug delivery system was used as a carrier for a bioactive combination (acetyl-carnosine and nicotinamide). A single intravitreal injection of the bioactive bi-composite system was able to dissolve early stage cataract within minutes of administration and was able to delay onset and progression of cataract in the preventative group of the study. Although this positive effect was observed, the cataract returned in 24-48 hours, which lead to the hypothesis that continuous use or multiple administrations of the system may derail cataract onset and progression. Although signs of cataract development were observed in the preventative group, some structures such as the cornea still remained clear for prolonged periods of time (14 days) as compared to the group that was only administered the cataract-inducing agent.

An alternative drug delivery approach that was assessed was the use of a contact lens-like device. The short-comings experienced such as the inability to obtain the correct shape and surface properties without the proper instrumentation, and inability to assess important properties such oxygen permeability in the absence of the instrumentation lead to its exclusion during the *in vivo* studies to prevent causing unnecessary harm and discomfort to animals.

TABLE OF CONTENTS

DESIGN AND DEVELOPMENT OF AN ELUTING-SUPRAMOLECULAR ASSEMBLED CONTACT LENS-LIKE DEVICE FOR THE TOPICAL BIOGENIC TREATMENT OF CATARACT ……i

DECLARATION ii

ACKNOWLEDGEMENTS iii

DEDICATION iv

RESEARCH OUTPUTS v

PRESENTATIONS vi

ANIMAL ETHICS CLEARANCE vii

PATENT APPLICATION viii

ABSTRACT ix

TABLE OF CONTENTS x

LIST OF FIGURES xviii

LIST OF TABLES xxiv

LIST OF EQUATIONS xxv

CHAPTER 1 1

INTRODUCTION 1

1.1. Background to the Study 1

1.2. Statement of the Problem 1

1.3. Cataract as an Example of Oxidative Stress-Based Disease and Treatment Strategies 2

1.4. Rationale for the Development of a *In situ* ocugel system embedded with optimized nanosponges complexes for Ocular Drug Delivery and Ternary Peptide-based Combination for the Prevention and Treatment of Cataract 5

1.5. Novelty of the Study 10

1.6. Aim and Objectives of this Study 10

1.7. Overview of this Dissertation 11
1.8 References 12
CHAPTER 2 15
LITERATURE REVIEW: AN OVERVIEW OF CYCLODEXTRINS AND MULTI-CYCLODEXTRIN SUPRAMOLECUAR ENTITIES FOR ADVANCED DRUG DELIVERY APPLICATIONS 15
2.1. Introduction 15
2.2. Cyclodextrin Properties that Confer Suitability in Drug Delivery Applications 15
2.3. Types of Multi-Cyclodextrin Entities: Properties and Applications 19
2.3.1. Cyclodextrin-based Polyrotaxanes 20
2.3.2. Cyclodextrin-based Nanosponges 20
2.3.3. Cyclodextrin Polymers 22
2.4. Approaches to the Production of Cyclodextrins: Synthesis and Derivatives 22
2.4.1. Cyclodextrin-based Polyrotaxanes 23
2.4.1.1. The Threading Process 23
2.4.1.2. Rotaxanation of Pseudopolyrotaxanes 23
2.4.2. Cyclodextrin-based Nanosponges 24
2.4.3. Cyclodextrin Polymers 25
2.5. Cyclodextrins and Multi-Cyclodextrins Applied for Topical and Targeted Drug Delivery 25
2.5.1. Tissue Surface Applications 27
2.5.1.1. Ocular Surface Applications 27
2.5.1.2. Oral Cavity and Tooth Surface Applications 31
2.5.1.3. Dermal Applications 31
2.5.2. Mucosal Membrane Applications 32
2.5.2.1. Buccal Mucosa 33
2.5.2.2. Sublingual Mucosa 33
2.5.2.3. Nasal Mucosa 34
2.5.2.4. Pulmonary Mucosa 34
2.5.2.5. Rectal Mucosa 35

2.6. Potential Limitations of Cyclodextrins and Multi-Cyclodextrins in Topical Drug Delivery Applications 36

2.7. Concluding Remarks 36

2.8. References 36

CHAPTER 3 44

ESTER-BASED HYDROPHILIC CYCLODEXTRIN NANOPSPONGES AS TOPICAL OCULAR DRUG DELIVERY SYSTEMS 44

3.1. Introduction 44

3.2. Materials and Methods 47

3.2.1. Materials 47

3.2.2.1. Synthesis of Cyclodextrin-based Nanosponges 48

3.2.2.2. Preparation of Drug-Nanosponge Complexes 49

3.2.2.3. Binary Physical Mixture Preparation 50

3.2.3. Characterization of the Physicochemical Properties of the Nanosponges 50

3.2.3.1. Scanning Electron Microscopy for the Detection of the Morphological Characteristics of the Nanosponges 50

3.2.3.2. Fourier Transform Infra-red Spectroscopy for Detection of Polymer Functionalities in Nanosponges and Nanosponge-drug Complexes 50

3.2.3.3. Thermal Analysis by Differential Scanning Calorimetry 51

3.2.3.4. Crystallinity and Diffraction Pattern Detection 51

3.2.3.5. Determination of Size Distribution, Polydispersity Index and Zeta Potential of Nanosponges and their Complexes 51

3.2.3.6. Determination of the Complexation and Drug Loading Efficiency 51

3.2.3.7. Drug Release Determination from the Drug-nanosponge Complexes 52

3.2.3.8. Phase Solubility Testing Employing Diclofenac Sodium Salt as a Model Drug 52

3.2.3.9. Ex Vivo Corneal Permeation via Pig Eye Corneas 53

3.3. Results and Discussion 53

3.3.1. Characterization of the physicochemical properties of the nanosponge and drug-nanosponge complex 53

3.3.1.1. Nanosponge formation 53

3.3.1.2. Morphology assessment of the nanosponges 54

3.3.1.3. Thermal Analysis of the Nanosponges 57

3.3.1.4. X-ray Diffraction Analysis of the Nano-complex and Drug powder 59

3.3.1.5. Size Distribution, Polydispersity Index and Zeta Potential Analysis of the Nanosponges 60

3.3.2. Nanosponge Complexation and Drug Loading Efficiency 62

3.3.3. Drug Release Behavior from the Nanosponges 64

3.3.4. Phase Solubility Analysis for Determination of the Potential of the Complex to Function as a Solubilizing Agent 66

3.3.5. *Ex vivo* Corneal Permeation Employing a Pig Model 67

3.4. Concluding Remarks 68

3.5. References 69

CHAPTER 4 72

DESIGN AND OPTIMIZATION OF THE NANOSPONGES AND CHARACTERIZATION OF THE CONTACT LENS-LIKE DEVICE 72

4.1. Introduction 72

4.2. Materials and Methods 73

4.2.1. Materials 73

4.2.2. Optimization of the Drug-nanosponge Complexes Employing a Central Composite Design 73

4.2.3. Fabrication of the Contact Lens-Like Device (CLLD) 73

4.2.4. Textural Analysis and Bio-adhesion Studies on the CLLD 74

4.2.5. Scanning Electron Microscopy of the Nanosponges and CLLD 74

4.2.6. Size Distribution Measurement of the Nanosponges 74

4.2.7. Thermal Analysis of the Nanosponges 74

4.2.8. Detection of Polymer Functionalities Present in the Nanosponges and CLLD.....75
4.2.9. Drug Loading, Release and *Ex vivo* Permeation from the CLLD.....75
4.3. Results and Discussion.....76
4.3.1. Morphology, Size Distribution and Optimization of the Nanosponges.....76
4.3.2. Visual and Scanning Electron Microscopy Imaging of the CLLD discs.....77
4.3.3. Polymer Functionality Analysis of the Pure CLLD and Drug-Loaded CLLD.....78
4.3.4. Thermal Analysis of the Drug-Free CLLD and Drug-Loaded CLLD.....79
4.3.5. Drug-Loading, Complexation, Release and *Ex Vivo* Permeation from the CLLD.....79
4.4. Concluding Remarks.....81
4.5. References.....82
CHAPTER 5.....84
A MODICATION OF A STIMULI-SENSITIVE SOL-GEL SYSTEM USING OPTIMIZED NANOSPONGE COMPLEXES FOR OCULAR DRUG DELIVERY.....84
5.1. Introduction.....84
5.2.1. Materials.....86
5.2.2. Preparation of the Nanosponge *In Situ* OcuGel System.....86
5.2.3. Determination of the Thermo-gelation Point of the *in situ* ocugel systems.....87
5.2.4. Comparative Studies between the Solution and Gel state of the *In situ* ocugel system … 87
5.2.4.1. Determination of Alterations in Polymer Functionality.....87
5.2.4.2. Viscosity Transitions of the Gelling System.....87
5.2.4.3. Bio-adhesion Studies.....87
5.2.4.4. Bioactive/drug Release Studies from the Gelling System.....87
5.2.5. Sterilization Procedure.....88
5.2.6. Epi-ocular Eye Irritation Test.....88
5.2.7. *In Vivo* Administration of the *In* situ ocugel system embedded with optimized nanosponges complexes in New Zealand White Rabbits.....89

5.2.8. Ultra-Performance Liquid Chromatography Analysis for Quantification of Bioactive Content....90

5.3. Results and Discussion....90

5.3.1. Gelation Phenomenon of the *In Situ* Gelling System....91

5.3.1.1. Evaluation of the thermo-gelation point of the Nanosponge In Situ OcuGel System....91

5.3.1.2. Analysis of Polymer Functionality of the In Situ Gelling System 92

5.3.1.3. Visco-elastic Analysis of the In Situ OcuGel System 93

5.3.1.4. Bio-adhesive Strength of the In Situ OcuGel System....96

5.3.2. Polymer Functionality Analysis of the *In Situ* OcuGel System....97

5.3.2.1. Acetyl-carnosine-loaded *in situ* ocugel systems 97

5.3.2.2. Nicotinamide-loaded In Situ OcuGel Systems 98

5.3.3. Thermal Behavior of *In situ* ocugel system embedded with optimized nanosponges complexes 99

5.3.4. Bioactive release data from various *In situ* ocugel system embedded with optimized nanosponges complexes....100

5.3.5. Epi-ocular Eye Irritation Test correlation of *In Vitro* to *In Vivo* Results....102

5.3.6. Acetyl-carnosine and nicotinamide bioavailability in the aqueous humor, lens and vitreous humor following intravitreal and topical administration of the formulation 104

5.3.6.1. Acetyl-carnosine levels....104

5.3.6.2. Nicotinamide levels 106

5.4.1. *In situ* ocugel system embedded with optimized nanosponges complexes....109

5.4.2. Relation of the *in vitro* to *in vivo* behavior 110

5.5. Concluding Remarks....110

5.6. References....110

CHAPTER 6....113

IN VIVO ASSESSMENT OF THE BIOACTIVE ELUTING SUPRAMOLECULAR-ASSEMBLED *IN SITU* GELLING SYSTEM IN CATARACT-INDUCED RABBITS 113

6.1. Introduction 113
6.2. Materials and Methods 115
6.2.1. Materials 115
6.2.2. Preparation of the Bioactive Supramolecular-assembled *In Situ* Gelling System 115
6.2.3. Animal Ethics Considerations 115
6.2.3.1. Housing and Grouping 115
6.2.4. Induction and Treatment of Cataract in the Rabbit Eye Model 116
6.2.4.1. Preparation of Diquat Dibromide Solution 116
6.2.4.2. Induction of Cataract 116
6.2.4.3. Confirmation of Cataract Development 116
6.2.4.4. Prevention and Treatment of Cataract via the Bioactive Eluting Supramolecular-assembled In Situ Gelling System 116
6.2.5. Histopathological Examination of Ocular Tissues (Cornea, Lens and Retina) to Confirm Cataract Development 117
6.2.6. Sampling of the Aqueous Humor, Lens and Vitreous Humor 117
6.2.7. Assaying for Cataract Markers 117
6.2.7.1. Method for Glutathione Determination 117
6.2.7.2. Determination of the Total Nitrite 118
6.2.7.3. Determination of Malondialdehyde 118
6.3. Results and Discussion 118
6.3.1. Confirmation of Cataract Development 118
6.3.2. Levels of Cataract Markers in a Normal Eye and in a Cataractous Eye 120
6.3.3. Therapeutic Effects of the Bioactive Supramolecular-assembled *In Situ* Gelling System 122
6.4. Concluding Remarks 123
6.5. References 124
CHAPTER 7 126

CONCLUSIONS AND RECOMMENDATIONS 126

7.1 Conclusions 126

7.2. Recommendations 126

APPENDICES……128

1. APPENDIX A1: Research Paper 1 Abstract……128

2. APPENDIX B1: Podium Presentation Abstract (Sandton Conference)……129

3. APPENDIX B2: Poster Presentation Abstract (Postgraduate cross-faculty symposium)……130

4. APPENDIX C1: Ethics Clearance Certificate and Modifications and Extensions Documents……..131

LIST OF FIGURES

Figure 1.1: The eye structure showing the anterior and posterior segment components [1]. 1

Figure 1.2: A normal clear lens (bottom) and a cataractous lens (top)..... 3

Figure 1.3: Chemical structures of (a) Acetyl-carnosine and (b) carnosine molecules. 4

Figure 1.4: Chemical structure of nicotinamide depicting its 6-atom heretoarene (nitrogen-substituted aromatic ring) and peptide/amide bond (NHCO). 4

Figure 1.5: (a) β-cylodextrin and (b) pyromellitic dianhydride. 7

Figure 1.6: The bioactive *in situ* OcuGel system showing interactaction of all components……9

Figure 2.1: Schematic representation of α, β and γ- cyclodextrins depicting the number of rings as per cyclodextrin type, the outer hydroxyl (OH) groups, the inner oxygen (O) bridges, hydrogen (H) atoms and an estimation of the internal diameter [17, 18].....17

Figure 2.2: Schematic representation of a) Inclusion complex b) Host-guest chemistry of inclusion complex formation: Skeleton modified cyclodextrin in order to fit the size and shape of guest molecule [25, 26]. 18

Figure 2.3: Diagrammatic representation of the nomenclature of cyclodextrin-based pseudopolyrotaxanes (a: single ring-like cyclodextrin molecule threaded on a linear compound and b: multiple ring-like cyclodextrin molecules threaded on a linear compound) and polyrotaxanes (c, d & e: one, two and multiple ring-like cyclodextrin molecules threaded on a linear compound capped with a stopper molecule to prevent de-threading)……..24

Figure 2.4: a) Image depicting association of cyclodextrin molecules in the presence of a crosslinker (PDA- Pyromellitic dianhydride) to form a multi-cyclodextrin entity known as nanosponges [54], b) SEM (Scanning electron microscope) image showing the porous nature of nanosponges and c) TEM (Transmission Electron Microscope) image showing spherically shaped CDNS……25

Figure 2.5: Figure showing the diagrammatic representation of the pathway taken by cyclodextrin complexes after topical administration to biological membranes. Biological membranes have components such as phospholipids and cholesterol; their locations are the primary sites of entry the drug. The cyclodextrin extracts these components to form inclusion complexes which remain in the extracellular space until excretion takes place. The drug diffuses in through these new temporary pores. Drug diffusion also occurs through membrane transport pathways……26

Figure 2.6: Showing HET-CAM test result of a) γ-CD: HPMC nano-gel, b) negative control (saline) and c) positive control (0.1N NaOH). The test gave an irritation score close to zero for the nano-gel and negative control, confirming that the nano-gel is non-irritant [62]……..28

Figure 2.7: Diagrammatic representation of mechanism of permeability enhancement exerted by cyclodextrin-based drug delivery systems. A reversible inclusion complex of a cyclodextrin and drug molecule forms, upon topical administration to the eye, the CD extracts phospholipids and cholesterol from the biological membrane which opens up temporary pores that function as sites of entry for the drug molecules, while the CDs themselves are excreted through the uveoscleral
pathway……29

Figure 3.1: Schematic representation of the synthesis reaction for CDNS demonstrating the linkage of CD molecules by PMDA (crosslinking reaction)……..48

Figure 3.2: Representation of the morphological characteristics of the nanosponges. SEM images are showing the different shapes of the particles (a and b), that is, rod-shaped particles produced from lyophilization (a) and sphericals synthesized by spray drying (b) and images (c and d) show the porous surface nature as affected by crosslinker amounts (c and d)……55

Figure 3.3: Fourier spectra of (a) β-cyclodextrin (black), (b) pyromellitic dianhydride (red), (c)1:1 β-cyclodextrin to pyromellitic dianhydride nanosponges (blue), (d)1:2 β-cyclodextrin to pyromellitic dianhydride nanosponges (purple) and (e) 1:4 β-cyclodextrin to pyromellitic dianhydride nanosponges (green) from top to bottom, respectively……56

Figure 3.4: FTIR spectra of 1:2 drug with: (a) 1:1 CDNS (black), (b) 1:2 CDNS (red), (c) 1:4 CDNS (blue) and (d) diclofenac sodium salt alone (purple). These spectra show the differences in complexes in the presence of drug……57

Figure 3.5: Differential Scanning Calorimetry thermograms of, 3.5A: (a) β-cyclodextrin, (b) pyromellitic dianhydride and (c) nanosponges, and 3.5B: (a) β-cyclodextrin (black), (b) pyromellitic dianhydride (red), (c) 1:1 CDNS (pink), (d) 1:2 CDNS (green) and (e) 1:4 CDNS (purple). These thermograms show that crosslinking has occurred by the change in the thermal behavior and improved stability of the newly made nano-systems……58

Figure 3.6: DSC thermograms of the complexes (a) C2 (black), (b) C4 (red), (c) C6 (blue) and (d) diclofenac sodium salt (green). At the melting point of the drug (283-285˚C), the complex is not showing any signs of a melt process, but rather a crystallization process is taking place right before the melting point of the drug……...59

Figure 3.7: Diffractograms of the C2 (red), C4 (blue), C6 (green) complexes and diclofenac sodium salt (brown)……..60

Figure 3.8: Typical size distribution curves for the nanosponges (a) 1:2 CDNS and (b) 1:4 CDNS……..60

Figure 3.9: Drug release data for C2 and C4 complexes formulated from the 1:2 and 1:4 β-CD to PMDA CDNS. Significant drug levels were released within the first hour. Over 80% of the drug was in solution within this time. This was caused by the high solubility of the drug in aqueous solutions as imposed by the drug-solubilizing effects of the nanosponges……65

Figure 3.10: Graphical depiction of the universally proven function of cyclodextrins and their hydrophilic derivatives, as solubility enhancers. Increasing the nanosponge amount increased the amount of drug in solution (a). This also shows the A-P type complex behavior of drug solubility (b)……66

Figure 3.11: Drug permeation from the nanosponge complexes……68

Figure 4.1: (a) Spherical nanosponge-drug complexes collected using the nano-spray drying process and (b) Nano-size distribution range. 76

Figure 4.2: (a) Photograph of the devices prepared from polymer mixture and (b) SEM image of the device (due to the transparent nature, the device was reflecting light under SEM). The image is taken at magnifications above 6000x……77

Figure 4.3: FTIR spectra of (a) Drug (b) CLLD and (c) CLLD in form of polymer films fabricated from conventional hard contact lens polymers loaded with drug……..78

Figure 4.4: DSC thermograms of (a) drug, (b)CLLD in form of polymer films fabricated from conventional hard contact lens polymers and (c) CLLD loaded with drug……79

Figure 4.5: Drug release data of CLLD and nanosponges performed using the franz diffusion apparatus and also indicative of permeation……80

Figure 5.1: Chemical structure of acetyl-carnosine (a) and carnosine (b). The acetyl group in 1(a) is COCH3. Both molecules are composed of the two peptide bonds (dipeptide), 5-atom heteroarene and carboxylic acid groups……85

Figure 5.2: Chemical structure of nicotinamide depicting its 6-atom heretoarene (nitrogen-substituted aromatic ring) and peptide/amide bond (NHCO)……86

Figure 5.3: A DSC thermogram showing onset of gelation for the various gel concentrations 15%v/v (c), 20%v/v (a) and 25%v/v (b) formulations. A thermo-gelation point as low as 16°C was observed for the 25%v/v gel concentration. The arrow ↓ indicates the point of gelation for each in situ gelling system……92

Figure 5.4: FTIR spectra of the various gel concentrations, that is, 15% (a), 20% (b) and 25% (c).……93

Figure 5.5: Rheograms showing typical viscometric behavior of the drug delivery system under different stimuli. (a) and (b) show the generalized pattern of behavior in differences of viscosity measured in pascals for a l gel-from (purple) and liquid form (blue) of the gel systems, (a) shows the viscosity against time (seconds) as the measurement is carried out while (b) shows the viscosity against the inverse of time (per second), (c) shows the effects of loading nicotinamide, that is, an increase in overall viscosity and (d) shows the change in viscosity with temperature where a maximum viscosity is reached at physiological temperature range. ……94-95

Figure 5.6: FTIR spectra of (a) 15% (black), (b) 20% (red), (c) 25% (blue) acetyl-carnosine loaded ocugels and (d) pure acetyl-carnosine (purple)……98

Figure 5.7: FTIR spectra of (a) 15% (black), (b) 20% (red), (c) 25% (blue) nicotinamide-loaded ocugels and (d) pure nicotinamide .(purple)……..99

Figure 5.8: Thermogram highlighting thermal behavior of the *In situ* ocugel system embedded with optimized nanosponge complexes ……..100

Figure 5.9: Fractional drug release for acetyl-carnosine (a) and nicotinamide (b) from 15%v/v, 20%v/v, and 25% v/v *in situ* ocugel system embedded with optimized nanosponges complexes. Figure (c: 15%v/v), (d: 20%v/v) and (e: 25%v/v) show the fractional drug release from systems

containing both acetyl-carnosine and nicotinamide……101

Figure 5.10: Determination of ET-50 value for the formulation 103
Figure 5.11: Acetyl-carnosine levels in the aqueous humor, vitreous humor and lens of both the left and right eyes following formulation administration via the intravitreal and topical routes. (a), (b) and (c) show the bioactive content following intravitreal injection of 0.1mL, 0.2mL and 0.3mL of the *in situ* ocugel system, respectively, sampled at one week. The bioactive content following topical administration route sampled at one week and after 5 hours is shown by (d) and (e), respectively. 105
Figure 5.12: Nicotinamide levels in the aqueous humor, vitreous humor and lens of both the left and right eyes following formulation administration via the intravitreal and topical routes. (a), (b) and (c) show the bioactive content following intravitreal injection of 0.1mL, 0.2mL and 0.3mL of the *in situ* ocugel system, respectively, sampled at one week. The bioactive content following topical administration route sampled at one week and after 5 hours is shown by (d) and (e), respectively. 107
Figure 5.13: This is a depiction of anterior elimination in the aqueous humor flow (1) (A) and posterior clearance of drugs through blood-ocular barriers after an intravitreal injection (B). The drug permeates through the posterior iris epithelium into iris vein and is drained by vortex veins (2), through the non-pigmented ciliary epithelium to ciliary muscles and from the ciliary plexus to the episcleral veins (3), to the retinal capillaries and through the retinal pigment epithelium (RPE) into the choroid and systemic circulation (4) [17]…….108

Figure 6.1: Depiction of an intravitreal injection……117

Figure 6.2: Cataract progression from onset to maturity. This shows the process of cataract induction following a single injection. (a) Normal clear lens, (b) Early stage cataract with scattered opacity, (c) a more prominent cataract opacity and (d) mature dense cataract covering the entire lens. ……..119

Figure 6.3: Histomorphological examination of a normal eye and a cataractous eye to confirm cataract development (IDEXX labs): (a) and (b) represent a normal and cataractous lens, respectively, at 40x magnification, while (c) and (d) represent normal and cataractous retinal tissue, respectively captured at 40x magnification. After treatment with cataract, during the stage when the cataract is cleared, (a) and (c) would correspond with the histomorphological

examination while when cataract returns 48 hours later, (b) and (d) would correspond with those results. 120
Figure 6.4: Images showing (a) normal eye, (b) the presence of early stage cataract and (c) 30 minutes after treatment with the *in situ* ocugel system. 123

LIST OF TABLES

Table 2.1: Characteristic features of α, β and γ- cyclodextrins 16
Table 2.2: Examples depicting the role of cyclodextrins in improving the properties of different compounds for delivery to the body……...19

Table 2.3: Cyclodextrin formulations currently on the market [56, 57]……26
Table 2.4: Applications of CDNS and PRs and potential benefits to ocular drug delivery……...30
Table 3.1: List of the drug-nanosponge complexes synthesized along with the composition explained. 49
Table 3.2: Dynamic light scattering data of the drug-nanosponge complexes showing the varying zeta potential values as an indication of the stability of each complex formed……61
Table 3.3: Calculated drug loading and complexation efficiencies of the different complexes……..63
Table 4.1: Composition of the contact lens-like device (CLLD)……...74
Table 4.2: Design data for the 13 formulations of the FC-CCD which lead to the production of the optimized formulation……..77

Table 5.1: Contents of the formulations employed for drug release analysis and subsequent *in vivo* studies 88
Table 5.2: Thermal behavior of the *in situ* gelling systems.....91
Table 5.3: Textural analysis studies showing the maximum detachment force which is representative of the bio-adhesive strength of the system..... 96-97
Table 5.4: Percentage (%) viability of the EpiOcular Tissue Model (OCL-200) at a particular exposure time to formulation 102
Table 6.1: Levels of cataract markers in cataractous and normal eye states 121

LIST OF EQUATIONS

Equation 1.....52
Equation 2.....52
Equation 3.....102