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A BIORESPONSIVE INJECTABLE OCUGEL FOR POST-SURGICAL CATARACT TREATMENT

Noko Jacqueline Lekoloane

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

Master of Pharmacy



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2022

DECLARATION

I, Noko Jacqueline Lekoloane (2287738), a student registered for the Master of Pharmacy in the Faculty of Health Sciences at the University of the Witwatersrand, Johannesburg, South Africa in the academic year 2022.

Hereby declare the following:

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 Prior to this, this thesis has not been submitted for the attainment of any degree or examination at this or any other university.

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Signature:  Date: 28 June 2022

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DEDICATION

This dissertation is dedicated to my family more especially my mother, Kwena Violet Lekoloane. My family's unwavering support throughout my seemingly never-ending academic journey, has allowed me to pursue my dreams without boundaries.

RESEARCH OUTPUTS

Research outputs at conference proceedings

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Lekoloane, N.J., du Toit, L.C., Kondiah, P.P., Makgotloe, A., Choonara, Y.E. (2022). *In Vivo* Investigation of the Dual Drug-Loaded Thermoresponsive thermo-ocugel for Potential Treatment of Post-Cataract Surgery Infection and Inflammation. (To be submitted) (Appendix D)

ABSTRACT

The eye is a complex organ with a unique anatomy and physiology. Topical formulations are still the most widely preferred non-invasive route of drug administration used to treat ocular diseases. This is owing to their ease of administration and high patient compliance. Nonetheless, the use of these conventional ocular formulations present bioavailability drawbacks as a result of the numerous anatomical constraints such as tear turnover, and static ocular barriers (sclera, different layers of the cornea, blood-aqueous and blood-retinal barriers) and dynamic ocular barriers (tear dilution, conjunctival blood flow, choroidal blood flow, and lymphatic clearance). These barriers pose a challenge and impede deeper ocular drug permeation. As a result, only around 5% of the topically administered dose penetrates deeper eye tissues. To overcome these ocular drug delivery barriers and improve ocular bioavailability, tremendous effort has gone into ocular research to develop safe and patient-friendly novel drug delivery systems/devices/biomaterials, such as emulsions, ointments, suspensions, nanomicelles, nanoparticles, liposomes, dendrimers, implants, microneedles, contact lenses, nanosuspensions, and *in situ* thermosensitive gels. The design of these drug delivery systems ranges from employing natural/synthetic polymers, peptides/proteins and ocular bioactives for treatment and management of ocular diseases.

There have been great advancements in the area of ocular drug delivery. However, there is still no system that meets all the requirements of an ideal ocular drug delivery system. The goal of this research was to develop a stimuli-responsive intracamerally-injected thermo-ocugel system for post-cataract surgery infection and inflammation treatment or prophylaxis. A dual drug-loaded *in situ*-forming injectable thermoresponsive chitosan-gelatin hydrogel was developed employing naturally-derived polymers and crosslinkers that would aid cytocompatibility and biocompatibility in terms of presenting optimal mechanical properties, in combination with the anti-inflammatory agents such as dexamethasone sodium phosphate (DSP), and anti-bacterial agents such as moxifloxacin hydrochloride (MOX) The properties of the ocular drug delivery system were explored by employing double crosslinking approach to fabricate an ocular system capable of forming an *in-situ* hydrogel at ocular physiological conditions. The double crosslinking approach enabled the dual incorporation of MOX and DSP, in addition to forming the *in situ* thermoresponsive hydrogel. This drug delivery system acted as a reservoir for both MOX and DSP to prolong their residence time and control their release. thus to decreasing the need for frequent administration.

The drug delivery system exhibited thermoresponsive behaviour with gel formation occurring within a temperature range of 25 to 38 °C with the highest gelling point at 32 °C. Rigidity (G') of this system started increasing from 5 hours of genipin crosslinking and reached 3.5 kPa in 12 hours. The system allowed for dual drug encapsulation efficiencies of 91.48% and 75.27%, and drug loading of 1.87% and 6.14% for DSP and MOX, respectively. The system showed a burst release of 52.75 ± 3.12%, and 60.94 ± 8.31% in 12 hours, which increased to 76.61 ± 5.91% and 81.71± 4.85% in 3 days, followed by a steady release of 0.53 ± 0.15 % and 0.81 ± 0.26% per week for DSP and MOX, respectively. The system showed a rapid fluid uptake of 126.02% in the first 3 days followed by a gradual increase up to 198.72% in 28 days. The system exhibited a low level of erosion (1.5%) on the first 3 days whereas a slightly higher degree of erosion rate (16.58%) was observed between 3 and 14 days and the overall surface erosion was 18.20% in 28 days. The dual drug incorporation, fluid uptake and surface erosion properties of the fabricated novel thermo-ocugel system were suitable for the application as potential treatment of post-cataract surgery infection and inflammation.

The thermo-ocugel's *in vivo* performance and safety was evaluated in order to provide more evidence of its ocular compatibility and drug levels achieved in normal ocular conditions. Significant inflammatory alterations, as well as any differences in the ocular tissues as a result of the thermo-ocugel injection or the injection technique, were assessed via histological analysis. The thermo-ocugel introduction to ocular tissues following intracameral injection resulted in notable cases of inflammation. However, the inflammation cases showed great resolution within a period of 21 days for the dual drug-loaded thermo-ocugel. The high therapeutic concentrations (>0.2 µg/mL) attained in the first 7 days for MOX highlight that the drug level attained would be sufficient to induce a therapeutic antibiotic effect while still remaining under the reported non-toxic concentrations of 150 µg/mL for the required antibiotic treatment period of 7 days. The release profile for DSP indicated concentrations which were above the MEC (0.5164 µg/mL) for the 28-day period of investigation. The thermo-ocugel thus achieved therapeutic levels of both the antibiotic and anti-inflammatory agent over the required treatment periods, while significantly reducing administration frequency, which is one of the drawbacks associated with antibiotic and anti-inflammatory eyedrop formulations on the market for post-cataract surgery infection and inflammation. Furthermore, the prolonged maintenance of effective ocular anti-inflammatory drug concentrations is a good indication of the thermo-ocugel's efficacy in counteracting the pathological tissue changes that occur when inflammation is prompted following ocular surgical procedures.

CHAPTER ONE

Introduction and Rationale of the Study

1.1. Background of the study 1

1.2. Rationale and motivation of the study.....4

1.3. Aim and objectives 6

1.4. Novelty of the study and potential application of the biomaterials 7

1.5. Overview of the thesis.....7

CHAPTER TWO

Thermosensitive Injectable Hydrogels as Ocular Drug Delivery Systems for Postoperative Endophthalmitis

2.1. Introduction 11

2.2. Routes of Administration 13

2.3. Thermosensitive Hydrogels for Delivery of Ocular Drugs 16

2.3.1 Thermoresponsive Hydrogels for Delivery of Ocular Antibiotics 17

2.3.1.1. Approaches For Encapsulating and Delivering Ocular Antibiotics via the use of Thermoresponsive Hydrogels 19

2.3.2. Thermoresponsive Hydrogels for Delivery of Ocular Corticosteroids 22

2.3.3 Thermoresponsive Hydrogels for Delivery of Ocular Dual Drugs 26

2.4. Prospective Trends 28

2.4.1. Delivery of Hydrophobic Drugs 28

2.4.2. Intraocular Lens Therapy 29

2.5. Concluding Remarks 30

CHAPTER THREE

Fabrication of a dual drug loaded thermoresponsive chitosan-gelatin hydrogel for potential treatment of post-cataract surgery infection and inflammation

3.1. Introduction 44

3.2. Materials and Methods 46

3.2.1. Materials 46

3.2.2. Fabrication of thermoresponsive thermo-ocugel hydrogels 46

3.2.3. Evaluation of molecular and chemical characteristics of biomaterials via FTIR 47

3.2.4. Evaluation of the diffraction pattern of lyophilised hydrogels via XRD 47

3.2.5. Thermostability assessment of biomaterials and hydrogels 47

3.2.6. Assessment of mechanical properties of hydrogels 47

3.2.7. Analysis of drug loading and release from the thermo-ocugel 48

3.2.8. Evaluation of the surface morphology of the fabricated hydrogels 49

3.2.9. Fluid uptake and erosion of the thermo-ocugel 49

3.2.10. Kinetic analysis of drug release from the thermo-ocugel 49

3.3. Results and Discussion 50

3.3.1. Fabrication of a thermoresponsive hydrogel 50

3.3.2. Evaluation of molecular and chemical characteristics of biomaterials via FTIR 51

3.3.3. Evaluation of the diffraction pattern of biomaterials via XRD 55

3.3.4. Thermostability assessment of native components and hydrogels 56

3.3.5. Assessment of mechanical properties of hydrogels 58

3.3.6. Analysis of drug loading and release from the hydrogel 64

3.3.7. Morphological features of the lyophilized hydrogels 65

3.3.8. Fluid uptake and erosion of the thermo-ocugel 66

3.3.9. Relationship between cumulative release with fluid uptake and erosion of the thermo-ocugel 68

3.4. Concluding Remarks 70

CHAPTER FOUR

In vivo studies conducted on dual drug loaded thermoresponsive chitosan-gelatin hydrogel (thermo-ocugel) for potential treatment of post-cataract surgery infection and inflammation

4.1. Introduction 75

4.2. Materials and Methods 77

4.2.1. Materials 77

4.2.2. Analysis of the *In vivo* behaviour of the thermo-ocugel in Rabbits 78

4.2.2.1. Sterilisation of the thermo-ocugel for surgical preparation 78

4.2.2.2. *In vivo* study design 78

4.2.2.3. Histomorphological Analysis for assessment of the degree of ocular inflammation following the intracameral injection of the thermo-ocugel 79

4.2.3. Quantification of *In vivo* anti-inflammatory and antibiotic concentrations released from the thermo-ocugel into the ocular fluids of the anterior segment of the rabbit eye model under normal conditions 82

4.2.3.1. Preparation of calibration standards 82

4.2.3.2. Sample preparations for drug release analysis 82

4.3. Results and Discussion 83

4.3.1. Analysis of the *In vivo* behaviour of the thermo-ocugel in Rabbits 83

4.3.2. Histomorphological findings following intracameral injection of the thermo-ocugel and drug-free hydrogel in normal rabbit eye 85

4.3.2.1. Effects of the thermo-ocugel in the normal rabbit eye at day 3 85

4.3.2.2. Effects of the drug-free hydrogel and thermo-ocugel in the normal rabbit eye at day 7 86

4.3.2.3. Effects of the drug-free hydrogel and thermo-ocugel in the normal rabbit eye at day 14 87

4.3.2.4. Effects of the thermo-ocugel in the normal rabbit eye at day 21 88

4.3.2.5. Effects of the drug-free hydrogel and thermo-ocugel in the normal rabbit eye at day 28 89

4.3.3. Overall effect of the drug-free hydrogel and the thermo-ocugel in the eye.....90

4.3.4. Quantification of *In vivo* anti-inflammatory and antibiotic concentrations released from the thermo-ocugel into the ocular fluids of the anterior segment of the rabbit eye model under normal conditions.....91

4.4. Concluding Remarks.....93

CHAPTER FIVE

Conclusion and Recommendations

5.1. Conclusion 96

5.2. Recommendations.....98

APPENDICES

Appendix A: Animal Ethics Clearance Certificate 99

Appendix B: Review Paper 100

Appendix C: Research Paper 1 101

Appendix D: Research Paper 2 102

Appendix E: Turnitin Report ……..103

LIST OF FIGURES

Figure 1.1. Schematic representation of components of the eye 3

Figure 1.2. Schematic diagram outlining the thermogelling mechanism from the interconnected thermo-ocugel matrix 6

Figure 2.1. Major routes of ocular drug administration 13

Figure 2.2. Outline of minor, moderately used, and major groups of antibiotic classes for POE 17

Figure 2.3. Structural representation of polymer conjugation/copolymerisation of chitosan with monocarboxylated Pluronic F-127 in EDC/NHS (1), polymer-drug complexation of chitosan-moxifloxacin complex (2), and chemical crosslinking of chitosan with genipin (3).....22

Figure 3.1. Schematic representation of interactions between polymers and crosslinkers i.e.; Ionic interaction between CS NH groups and the phosphate groups from BGP (1), CS-BGP complex grafted onto GEL-MOX-DSP (2), Chemical crosslinking of CS-BGP-GEL-MOX-DSP by genipin (3), and A dual drug loaded thermoresponsive CS-GEL hydrogel (4).....51

Figure 3.2. (a) FTIR analysis of biomaterials. "J" indicates the region where the interactions affect the chemical structure. (b) XRD analysis of biomaterials 56

Figure 3.3. Thermo-stability properties of fabricated biomaterials. (a) Represents the DSC curves and (b) represents TGA thermograms of the biomaterials.....58

Figure 3.4. Rheological properties of the hydrogels. (a) thermo-responsive properties of the hydrogels and (b) shear storage modulus as a function of time for all hydrogels, and (c) time-lapse of the thermo-ocugel which assesses the interaction between the polymers and the crosslinkers.....61

Figure 3.5. Mechanical properties of the hydrogels (I) F1, (II) F2, (III) F3, and (IV) thermo-ocugel. (a) Represents the shear elastic modulus as a function of shear stress, (b) Shear viscosity and (c) shear elastic modulus and shear viscosity as a function of temperature of the hydrogels. 63

Figure 3.6. Cumulative release of DSP and MOX from the hydrogel under physiological conditions (37 C, PH 7.4).....65

Figure 3.7. SEM images of the fabricated hydrogels. (a) F1, (b) F2, (c) F3, and (d) thermo-ocugel 66

Figure 3.8. Erosion and swelling of hydrogel under physiological conditions and slightly agitated the samples via the use of the orbital shaker at 37 °C at a speed of 50rpm 68

Figure 3.9. (a) Cumulative release as a function of swelling and (b) cumulative release as a function of erosion of hydrogel under physiological conditions. The analysis were made at each of the same time points for each of the test. Erosion data sets were used from day 1 to day 28 which corresponded to the cumulative release 69

Figure 4.1. *In vivo* study outline (Note: Intracameral injection was undertaken on day 0. Euthanasia and enucleation occurred on days 0, 3, 7, 14, 21, and 28).....81

Figure 4.2. Photographic evidence of (a) rabbit housing (b) weighing (c) preparation for surgery with anesthetization (d) post-surgical care.....84

Figure 4.3. Consecutive images (a-d) depicting intracameral injection of the thermo-ocugel into the anterior segment of the rabbit eye 84

Figure 4.4. Images of the normal eye in the control rabbit which depicts normal appearance of the structures of the globe at 4x magnification 85

Figure 4.5. Histological slides at day 3 for the thermo-ocugel in the normal eye at 4x magnification 86

Figure 4.6. Histological slides at day 7 for the drug-free hydrogel (a) and thermo-ocugel (b) in the normal eye at 4x magnification 87

Figure 4.7. Histological slides at day 14 for the drug-free hydrogel (a) and thermo-ocugel (b) in the normal eye at 4x magnification 88

Figure 4.8. Histological slides at day 21 for the thermo-ocugel in the normal eye at 4x magnification 88

Figure 4.9. Histological slides at day 28 for the drug-free hydrogel (a) and thermo-ocugel (b) in the normal eye at 4x magnification 90

Figure 4.10. Graphical representation depicting the degree of cellular infiltration by lymphocytes, plasma cells, and mononuclear and polymorpho-nuclear leukocytes over time for control, thermo-ocugel and drug-free hydrogel injected eyeball samples.....91

Figure 4.11. Calibration curves derived for (a) MOX and (b) DSP in the presence of ciprofloxacin as an internal standard, in ocular fluids of the anterior segment. *In vivo* drug release profiles of (c) MOX and (d) DSP in normal rabbit eye.....93

LIST OF TABLES

Table 2.1. Benefits and obstacles of different ocular routes of administration 14

Table 2.2. Thermoresponsive hydrogels for delivery of ocular drugs for POE prophylaxis.....26

Table 3.1. Major FTIR bands of formulations and comparative transmittance of formulations vs. native materials.....51

Table 3.2. *In vitro* kinetics analysis of drug release from the thermo-ocugel.....66

Table 4.1. Drugs and recommended dosages as administered to rabbits.....77

LIST OF EQUATIONS

Equation 3.1. Drug loading % **45**

Equation 3.2. Drug release (pt) %..... ..... **46**

Equation 3.3. Cumulative release % **46**

Equation 3.4. Swelling %..... **47**

Equation 3.5. Erosion %..... **47**

Equation 3.6. Zero-Order model **47**

Equation 3.7. First-Order model **47**

Equation 3.8. Higuchi model..... **47**

Equation 3.9. Korsmeyer-Peppas model **47**

Equation 3.10. Hixson-Crowell cube root law **47**

LIST OF COMMONLY-USED ABBREVIATIONS

BGP - β-glycerophosphate disodium salt hydrate

BAB - blood-aqueous barrier

BRB - blood-retinal barrier

CS - chitosan

CSBGP - chitosan-β-glycerophosphate

CSBGPGEL - chitosan-β-glycerophosphate-gelatin

CSBGPGELGEN - chitosan-β-glycerophosphate-gelatin-genipin

CSBGPGELGENMOXDSP - chitosan-β-glycerophosphate-gelatin-genipin-moxifloxacinHCL-dexamethasone sodium phosphate

DSC - differential scanning calorimetry

DSP - dexamethasone sodium phosphate

FTIR - Fourier-transform infrared spectroscopy

GEL - gelatin

GEN - genipin

LMW - low molecular weight

MOX - moxifloxacin HCl

NSAIDs - nonsteroidal anti-inflammatory drugs

Thermo-ocugel - dual drug loaded thermoresponsive chitosan-gelatin hydrogel

PBS - phosphate-buffered saline

POE - postoperative endophthalmitis

SEM - scanning electron microscopy

STF - simulated tear fluid

TGA - thermogravimetric analysis

TASS - toxic anterior segment syndrome

XRD - x-ray diffraction

CHAPTER 1

Introduction and Rationale of the Study

1.1. Background of the study

The human eye is a complex organ with unique anatomical and physiological features. The structure of eye is sub-divided into 2 main chambers namely the anterior segment which accounts for approximately one-third of the eye and the posterior segment which accounts for two-third of the eye [1]. The anterior segment is composed of the cornea, conjunctiva, iris, ciliary body, lens, and aqueous humour, while the posterior segment includes the sclera, choroid, retina, and vitreous body (Figure 1.1) [2, 3]. The anterior and posterior segments of the eye may be affected by several vision-threatening diseases that carry the potential of imposing significant clinical burdens worldwide [4]. Cataract is among these vision-threatening diseases as it has been reported to account for approximately 50% cases of blindness in low-income countries and around 5% cases in developed countries (approximately 20 million people in the worldwide) [5]. Cataract surgery is relatively safe but carries the risk of post-operative infection and inflammation. In certain cases, tissue damage, intra-operative posterior capsular, and postoperative endophthalmitis can occur. Failure to address the post-operative infection and inflammation could result in vitreous loss or dropped nucleus which could further predispose the patient to oedema or retinal detachment risks [5]. During conventional ocular surgery prostaglandin levels rise [6]. These are intraocular modulators for inflammation and if not controlled could lead to complications and vision loss. The use of corticosteroids and/or non-steroidal anti-inflammatory drugs (NSAIDs) is required to inhibit the synthesis of prostaglandins [7]. Another factor contributing to post-surgical vision loss is an invasion of highly virulent bacteria such as a streptococcal infection that necessitates a course of an antibiotic for prophylaxis [8, 9].

The most commonly used and widely accepted ocular therapeutics are eye drops. Though they account for a large percentage of ocular therapies in the market, they still have drug release and bioavailability drawbacks [10]. Topically applied eye drops are prone to rapid drainage from the ocular surface thereby shortening drug retention and exposure. This in turn limits drug bioavailability thereby posing a tedious effort to stick to the desired and effective dose [11]. Other factors that hinder the bioavailability of ocular therapeutics include the static and dynamic ocular barriers which are in place to prevent toxic chemical substances to reach various tissues of the eye. The downsides to these barriers include the prevention of entry and diffusion of drug molecules to ocular tissues thereby significantly reducing drug duration [3].

The first form of obstacle in ocular local drug delivery is the tear film. This barrier consists of the outer lipid layer, aqueous middle layer, and mucin inner layer. This is where healing and infection control takes place as a result of a combination of electrolytes and numerous proteins working in unison. This film has a fast turnover and restoration time thereby results in quick drainage of topically administered drugs [12, 13]. The static barriers such as corneal -epithelium, -stroma, -endothelium, blood-aqueous barrier, sclera, and blood–retinal barrier and the dynamic barriers such as tear dilution, conjunctival blood flow, choroidal blood flow, and lymphatic clearance also hinder drug absorption which in turn results in reduced bioavailability of the administered dose where approximately <5% of the administered dose is absorbed [4, 14]. To circumvent these drawbacks, an ideal ocular drug delivery system should exhibit enhanced maintenance of therapeutic drug concentrations at the target site, improved bioavailability to aid in the reduction of dosage frequency, and avoidance of dynamic and static ocular barriers to overcome rapid elimination. Above all, the drug-delivery mechanism should cause no adverse ocular reactions and aim for improved drug bioavailability. To meet these requirements, extensive research has been conducted on various natural/synthetic polymer blends which include polysaccharides (chitosan, hyaluronic acid, and alginates) [15], and proteins (collagen and gelatin) [16]. The use of natural polymers in ocular drug delivery has gained attention due to their excellent biocompatibility, non-toxicity, biodegradability, and compliance to chemical modifications.

Chitosan represents one of the widely used polymers for ocular drug delivery due to its excellent mucoadhesion and evident presence of positively charged backbone at physiological pH. These charges allow for ease of chemical modifications which lead to improved rheological properties, tailored mucoadhesion, and increased ocular retention [17]. Apart from mucoadhesion, chitosan has been reported to show penetration-enhancing properties and increased cellular permeability in ocular tissues [18]. Another one of the most widely used biomaterials for ocular drug delivery due to its high-biocompatibility, better solubility, and less antigenicity is gelatin [19]. Various crosslinking strategies have been explored to develop chitosan-gelatin hydrogels for potential use in ocular drug delivery [20-22]. The most commonly used molecules for chemically crosslinking chitosan and gelatin include genipin and glutaraldehyde which have been reported to yield significant alteration of mechanical and biochemical properties. In addition, chitosan can also undergo ionic interactions with other polymers, and/or crosslinking moieties such as beta glycerophosphate [23].

The properties of these biomaterials (chitosan and gelatin), and crosslinkers (genipin and beta glycerophosphate), were explored in the current study for potential dual drug delivery of moxifloxacin hydrochloride and dexamethasone sodium phosphate. These biomaterials were

selected based on their desirable properties such as mucoadhesion, biocompatibility, and biodegradation which makes them great candidates for ocular drug delivery and tissue engineering [17, 19]. The inclusion of crosslinkers provide an increase in ocular drug retention time and improved efficacy. In addition, crosslinking aids in the fabrication of *in situ* forming and thermoresponsive hydrogels [22]. The use of moxifloxacin hydrochloride and dexamethasone sodium phosphate as the drugs of choice is to aid in the prevention of secondary infection and inflammation following cataract surgery.

Herein, the biomaterials discussed above will be explored to fabricate a stimulus-responsive ocular gel (thermo-ocugel) to be injected intracamerally directly following cataract surgery. The current study propose to employ the double crosslinking approach and evaluate the physicochemical, and physicomechanical properties along with the conduction of *in vitro* and *in vivo* analysis.

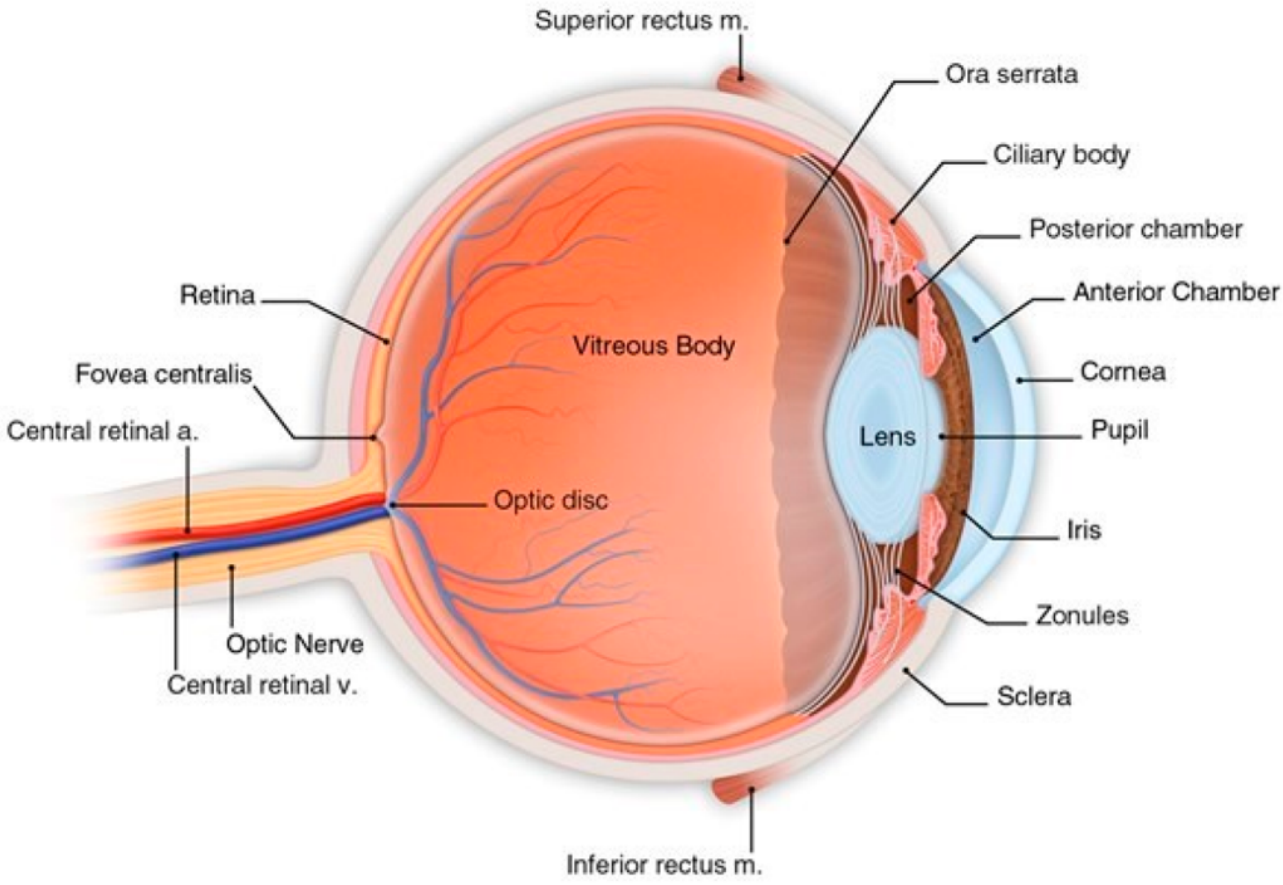

Figure 1.1. Schematic representation of components of the eye. Image obtained with permission from Petrillo *et al*. [24].

1.2. Rationale and motivation for the study

Post-cataract surgery antibiotic drops are employed to prevent infection in conjunction with corticosteroids and/or NSAIDs for the reduction or elimination of inflammation. Typically, moxifloxacin, vancomycin, or cefuroxime eye drops are used for 1-2 weeks, while dexamethasone or prednisolone containing eye drops are used for a period of 3-4 weeks [25]. The use of eye drops as prophylactic measures post cataract surgery is a vital component of the healing process. Patients require these eye drops for swift recovery. Therefore, incorrect use of eye drops such as premature blinking, abrupt discontinuation, and poor hygiene will result in failure to eliminate bacteria in and around the eyes resulting in infection, slowing of the healing process, and complications that could negatively impact vision. Furthermore, the major challenge associated with using eye drops post-cataract surgery is their short duration of action as a result of the static and dynamic ocular barriers. This requires frequent administration to achieve the desired effect and majority of the dose administered is wiped out by the constant blinking motion of the eye with only 20% (approximately 6 µL) of instilled dose retained and absorbed in the precorneal pocket [26]. Since cataract surgery can be performed on an outpatient basis, the onus is on the patient to ensure proper post-surgery care, and frequently instilling eye drops can be an inconvenience which may negatively affect patient adherence to treatment [27]. Reasons associated with non-adherence could include. but are not limited to, difficulty in coordinating the eye drops due to the already impaired vision. To address issues with patient compliance, there is a need for a long-term solution that could be applied at the point of surgery.

Several drug delivery systems have been developed over the years with the aim of addressing the above-mentioned drawbacks. However, they still present few limitations such as limited incorporation of multiple drugs, low patient acceptance, lack of bioresorbability and undesirable drug release profiles. An ideal drug delivery system for post-cataract surgery should exhibit the following performance attributes: (a) offer effective release of an antibiotic for a period of 1-2 weeks, and an anti-inflammatory drug up to 4 weeks, (b) allow for healing of the ocular tissues while maintaining the intraocular pressure, and (c) prevent secondary infections. Furthermore, there are properties that need to be enhanced or maintained in ocular drug delivery systems such as controlled biodegradation, adequate thermostability, and optimal viscoelastic properties. Only a few ocular drug delivery systems can currently meet the majority of these characteristics and performance requirements. As a result, there is a knowledge gap in the design of ocular drug delivery systems with optimum characteristics and performance.

This knowledge gap can be closed by designing and developing a biodegradable and bioresponsive intracamerally injectable thermo-ocugel applied directly following surgical removal of cataracts. The use of the intracameral route of administration would provide a solution to addressing disadvantages associated with traditional eye drops in terms of low bioavailability, short pre-corneal retention time, short term increase in intraocular pressure, rapid nasolacrimal drainage and frequent instillations [28]. An easily injectable system that forms a gel at body temperature in the intracameral space would be capable of maintaining ocular residence time and controlling intraocular pressure while ensuring effective concentrations at the targeted site for a minimum of 1 week for the antibiotic drug, up to 4 weeks.

The current study focused on the design and fabrication of a dual drug-loaded thermoresponsive chitosan-gelatin hydrogel for potential treatment of post-cataract surgery infection and inflammation. The application of naturally derived polymers and crosslinkers in combination with the anti-inflammatory and anti-bacterial agents would aid cytocompatibility and biocompatibility in terms of presenting optimal mechanical properties for use as an *in situ* forming injectable thermoresponsive hydrogel.

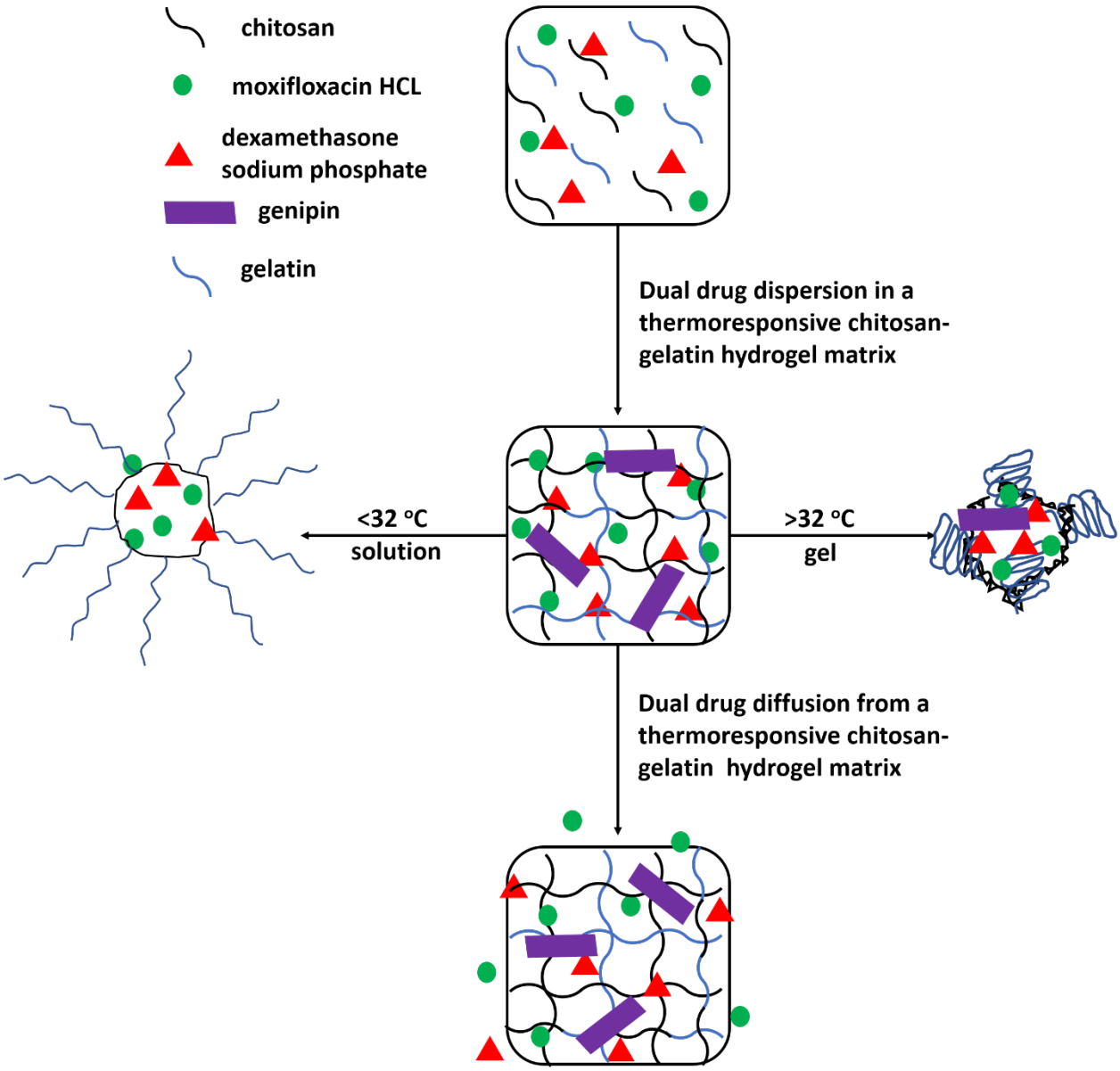

Figure 1.2. Schematic outlining the thermo-gelling mechanism from the interconnected thermo-ocugel matrix

1.3. Aim and objectives

The aim of the study was to develop a stimuli-responsive intracamerally-injected thermo-ocugel system for post-cataract surgery infection and inflammation treatment or prophylaxis. In order to attain the above-mentioned aim, the following objectives were undertaken:

1. To design bioresponsive hydrogel systems by employing suitable stimuli-responsive (thermo-responsive) polysaccharide networks and crosslinking approaches.
2. To analyze the physicochemical properties of the employed biomaterials and fabricated hydrogels.

3. To determine the physicomechanical properties of the hydrogels, specifically the viscoelastic transitions and *in situ* gelling capabilities in response to temperature.
4. To evaluate *in vitro* drug release at simulated ocular conditions.
5. To determine fluid uptake, degradation, and morphological properties of the thermo-ocugel over time.
6. To conduct *In vivo* studies on the rabbit eye model for analysis of biocompatibility, drug release behaviour, and prediction of the clinical potential of the thermo-ocugel.

1.4. Novelty of the study and potential application of the biomaterials

The novelty of this study lies within the use of double crosslinking approach to tailor for more desirable properties for controlled and site-specific delivery of drugs to ocular tissues. The double crosslinking approach allows for an ionic interaction which in turn enhance the thermoresponsitivity as well as chemical crosslinking. The crosslinking approach employed in this study is strategically designed to allow for the hydrogels to exist as a solution at room temperature and only transition into viscoelastic hydrogels *in situ* following ocular fluid exposure. The solution form of the hydrogels would allow for ease of administration especially in cases of injections. The hydrogels would be designed from natural polymers. The use of natural polymers was to improve the biocompatibility and biodegradability.

1.5. Overview of the thesis

Chapter 1: This chapter introduced the background of current research in the field of ocular drug delivery, highlighting the drawbacks associate with existing drug delivery systems and gaps in the field, all of which serve as the foundation for the study's rationale.

Chapter 2: This chapter outlined the employment hydrogels for optimal ocular drug delivery of antibiotics and corticosteroids. This chapter further provided a comprehensive review of the approaches employed in the hydrogel fabrication that affect property-performance factors. To that purpose, this chapter highlighted literature correlation between fabrication approaches and trends in the delivery of drugs to ocular tissues.

Chapter 3: This chapter addressed the previously identified gaps in the fabrication of appropriate and functional ocular drug delivery systems that can be modified for improved characteristics and optimal performance. Herein, a highly biocompatible, slowly biodegradable, dual drug loaded thermoresponsive chitosan-gelatin hydrogel is described. A

double crosslinking method is used for dual incorporation of MOX and DSP and also the *in situ* forming thermoresponsive hydrogel for enhanced mechanical properties, degradation, and *in vitro* dual drug release were among the assessed properties. The sustained drug release profile inferred that the thermo-ocugel would be ideal for post-operative infection and inflammation control.

Chapter 4: This chapter outlined the *in vivo* properties and performance of the thermo-ocugel. Furthermore, the thermo-ocugel's biocompatibility, delivery mechanism, and the likelihood of delayed toxic effects were evaluated against the control.

Chapter 5: This chapter outlined the outcome of the research along with prospective recommendations in the field of post-operative care and ocular drug delivery.

1.6. References

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CHAPTER 2

Thermosensitive Injectable Hydrogels as Ocular Drug Delivery Systems for Postoperative Endophthalmitis

2.1. Introduction

Postoperative endophthalmitis (POE) is one of the most serious complications of ocular surgery that presents in a form of an inflammatory reaction following intraocular procedures such as cataract surgery, keratoplasty, vitrectomy, and results in further visual impairment [1, 2]. The main causative organisms for POE have been attributed to virulent gram positive bacteria such as coagulase-negative staphylococci (i.e., *Staphylococcus epidermidis*), *Staphylococcus aureus* which is the most virulent [3], streptococci, enterococci, and gram negative bacteria such as bacilli [4]. These microorganisms act by colonizing the surface of intraocular tissues such as the eyelids, lacrimal sac, and the conjunctiva thereby causing infections [1]. Moreover, literature reports indicated that most POE cases present acutely, ranging from hours to a few days of surgery. These cases are treated as medical emergencies, as delays in treatment may result in permanent vision loss [1, 4].

The clinical symptoms present in these early stages include, but are not limited to decreased vision, red eye, eye pain, and hypopyon [4]. These symptoms could be attributed to preoperative risk factors such as blepharitis, and lacrimal duct obstruction. The intraoperative risk factors include inadequate disinfection of the eyelid, and inapparent ocular penetration. The postoperative risk factors includes wound leak, and vitreous incarceration in the surgical wound [1, 5]. In order to reduce the incidence of POE, each of the risk factors implicated in the pathogenesis should be addressed. This includes the employment of preoperative topical antibiotics and antiseptic agents, followed by the administration of subconjunctival antibiotics at the time of surgery [2]. In addition, a concomitant administration of an antibiotic and anti-inflammatory agents at the end of surgery as a prophylactic measure against POE showed better visual outcomes [6].

Antibiotic therapy in POE is the most effective in preserving vision. The use of these antibacterial agents work by inhibiting the synthesis of bacterial cell wall, altering bacterial cell membrane, inhibiting protein biosynthesis, or inhibiting bacterial nucleic acid synthesis [7]. In addition to antibiotic therapy, corticosteroids are drugs of choice due to their usefulness in eliciting numerous potent anti-inflammatory effects. For instance, they increase the synthesis of lipocortins that block phospholipase A_2 enzyme and stabilise mast cells in order to reduce

histamine release [8, 9]. Furthermore, the inhibition of this enzyme prevents the conversion of phospholipids to arachidonic acid, which is a critical step in the inflammatory cascade [9, 10]. Despite weaker evidence for efficacy, topical ocular formulations are still ubiquitous as POE prophylaxis [11, 12].

Topical eye drops are generally regarded as the most common and accessible route of administration. Nevertheless, they still have drug release and bioavailability drawbacks. These formulations are prone to rapid drainage from the ocular surface thereby shortening drug exposure which limits its bioavailability [13, 14]. The limited bioavailability poses a tedious effort for patients to stick to accurate dosing requirements leading to toxicity and further damage of ocular tissues [15]. Several attempts have been undertaken in the last decade to improve the efficacy of eye drops. A number of novel ocular drug delivery systems have been introduced as a result of these studies with the aim of improving drug bioavailability and retention time. Stimuli-responsive hydrogels, intraocular lens implants, and contact lenses are examples of this [16]. In particular, *in situ* forming, stimuli-responsive injectable hydrogels have gotten a lot of attention for POE. As a result, literature findings have shown that injectable administration of drug-loaded hydrogels can delay the instilled drug's fast removal, thus extending its retention period. [17, 18].

Stimuli-sensitive hydrogels are injected into the eye as a liquid and immediately turn into viscoelastic gels at the site of action, a process known as *in situ* formation. The sol-gel transition is triggered by a variety of stimuli, including temperature, pH, and ionic strength [19]. As a result, the *in situ* formed gel's residence period will be greatly increased. Furthermore, the encapsulated drugs will be released consistently throughout time, increasing bioavailability and lowering administration frequency [20]. There have been various *in situ* hydrogel systems proposed as innovative ocular dosage forms to improve the treatment of anterior segment problems, including but not limited to temperature-, pH-, and ionic-activated hydrogel systems. [21].

Thermosensitive hydrogels can prolong the precorneal residence time, and its formation by simple sol–gel transition at proper physiological temperatures. A literature report demonstrated that a thermosensitive double crosslinked chitosan-gelatin hydrogel formulation was successful in enhancing therapeutic effects of the loaded timolol maleate thereby reducing side effects [22]. These crosslinked sol-gel systems behave as liquid state when the ambient temperature is below low critical solution temperature (LCST) and form a gel when the environmental temperature is above the LCST. Herein, thermoresponsive hydrogel systems carrying antibiotics and corticosteroids were reviewed. Polymers used in fabricating

thermoresponsive hydrogels, different approaches of incorporating various drugs and properties of the hydrogels in relation to their application in POE will be discussed.

2.2. Routes of Administration

One of the most difficult areas of pharmaceutical research to date is the ocular route of drug delivery. This is due to the eye's complex architecture, physiology, and biochemistry, which makes it exceptionally resistant to external substances [23, 24]. Extensive studies have been conducted on multiple entry routes associated with the eye, through which ocular drugs may be delivered. These are divided into two main categories, namely, the anterior segment routes such as topical, periocular (i.e.; subconjunctival), and intracameral, and the posterior segment routes such as systemic, periocular, and intravitreal (Figure 2.1) [24]. Studies show that the success of therapeutic drug delivery through these routes depends on the targeted site and tissue barriers that could be encountered. Along with the type of pharmacological agents involved [25]. Each delivery route has its own benefits and obstacles [26]. Table 2.1 provides an overview of the different routes of ocular drug delivery along with their benefits and obstacles.

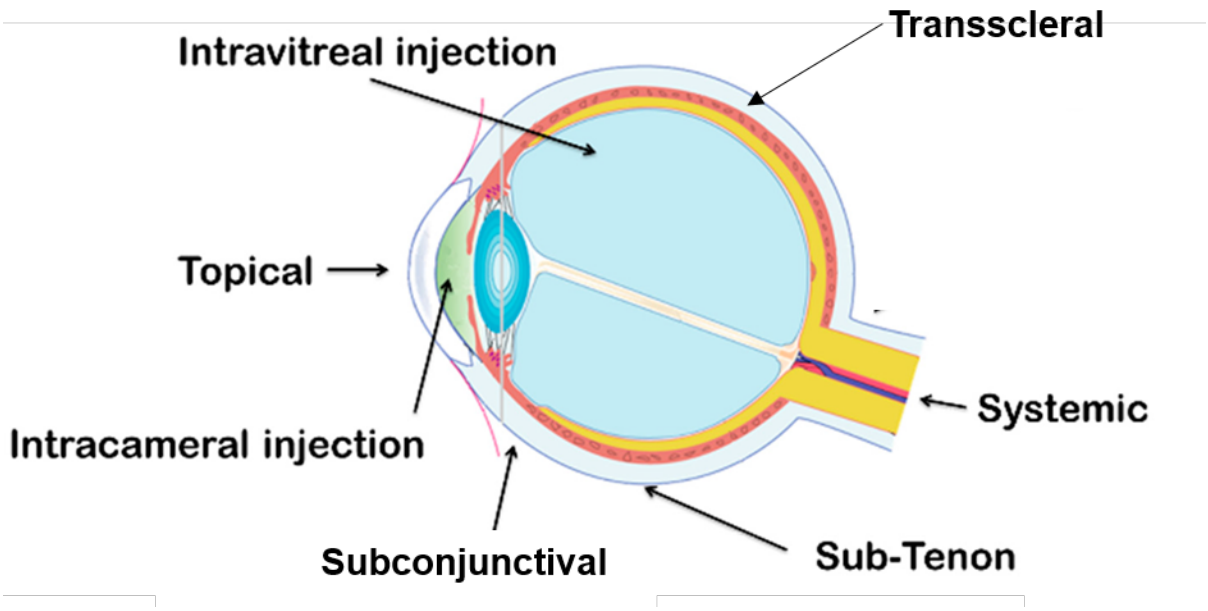

Figure 2.1. Major routes of ocular drug administration. Image adapted with permission from Dubald *et al*. [27]

Table 2.1. Benefits and Obstacles of Different Ocular Routes of Administration

Routes	Formulation	Benefits	Obstacles	Ref
Topical	• eye drops (solutions, suspensions) • emulsions • ointments	• Non-invasiveness. • Ease of use. • Low systemic circulation absorbance. • Avoidance of first pass metabolism.	• Limited entry into intraocular structures due to poor penetration through ocular barriers (i.e.; cornea, lacrimal drainage). • Poor delivery of macromolecules or drugs with molecular weight greater than 1kDA	[24, 26, 28, 30, 31]
Intravitreal	• Posterior segment injections	• Rapidly achieve therapeutic drug levels at the infection site by bypassing the blood-retinal barrier. • Provide the quickest and most direct route to attain therapeutic concentrations posterior segment.	• Most invasive. • Repeated injections could lead to potentially serious complications such as cataract, vitreous haemorrhage, retinal detachment, and endophthalmitis.	[26, 29,30,32]

Table 2.2. Benefits and Obstacles of Different Ocular Routes of Administration (ctd.)

Routes	Formulation	Benefits	Obstacles	Ref
Periocular (i.e.; subconjunctival, subtenon, and transscleral)	• Microneedles • Implants • injections	• Less invasive • Improve retinal drug bioavailability • Subconjunctival route offers the benefit of sustained drug release for both anterior and posterior segment as it surpasses conjunctival epithelial tight junctions • The sclera has a lower diffusion barrier, allowing for better drug absorption through numerous layers of ocular tissues.	• Drug clearance could be affected by high choroidal blood flow. • Scleral entry is only limited to drugs with molecular weight of less than 70 kDa.	[26, 28, 29, 30, 32, 33]
Systemic	• Oral, • IV injection	• oral approach is non-invasive • intravenous approach improves posterior segment drug efficiency	• High systemic doses are required to reach achieve therapeutic levels in the posterior segment, this could often yield potentially toxic effects. • Attainment of effective drug concentrations is a concern as most molecules are excluded by the ocular blood-neural barriers such as the anterior blood-aqueous barrier and the posterior blood-retinal barrier	[26, 28, 29, 30]

2.3. Thermosensitive Hydrogels for Delivery of Ocular Drugs

In recent years, significant progress has been made to modify existing natural and synthetic polymers and exploit their properties in the development of smart systems capable of releasing loaded drugs in response to ocular temperature. These biomaterials include, crosslinked natural polysaccharides such as cellulose and chitosan, and synthetic polymers such as polyethylene glycol, poly(*N*-isopropylacrylamide and Pluronic F-127 [34]. Herein biomaterials comprised of thermoresponsive polymers were reviewed for their application in delivering ocular bioactives. These targeted drugs include antibiotics and corticosteroids.

2.3.1 Thermoresponsive Hydrogels for Delivery of Ocular Antibiotics

The main causative organisms for POE are gram positive bacteria followed by gram negative bacteria, and only rarely fungi as highlighted in the details above. Therefore, the main aim of POE treatment or prophylaxis is to employ an antibiotic agent with a broad spectrum as soon as possible. The application of antibiotics that are incorporated in ocular hydrogels can be divided into minor and major groups.

The minor group of antibiotics consists of the less studied, explored or less used antibiotic classes which include macrolides, sulphonamides, and tetracyclines among others. Amphenicols and aminoglycosides form part of the moderately used classes which have been studied for potential use in POE treatment/prophylaxis and are still used due to their broad-spectrum activity. Amikacin which is a potent antibiotic for management of acute ocular infections and an aminoglycoside of choice due to its low cost and reliability against gram negative bacteria [35]. Other aminoglycosides that have been studied for use as prophylactic measures for POE include neomycin, tobramycin, and gentamycin [36]. However, toxicity and poor blood retinal barrier penetration posed to be a major concern for aminoglycosides especially when used intraocularly [37, 38].

The major group consists of well-studied POE prophylactic antibiotic classes which poses desirable properties. Amongst these, the most commonly used antibiotic classes include cephalosporins, fluoroquinolones, and glycopeptides [39]. Cefuroxime falls under the second generation of cephalosporins and is the most studied antibiotic for POE. In addition, ceftazidime which is the third generation of cephalosporins has equally been studied for POE [40]. This class of antibiotics work by disrupting the synthesis of the peptidoglycan layer of the bacterial cell wall thereby causing bactericidal activity [41]. The cephalosporins have activity

against both gram positive and negative bacteria, ranging from the first generation with greater activity against gram positive bacteria and the succeeding generations showing more of an increased activity against gram negative bacteria [42, 43]. Several *in situ* forming hydrogels have been employed in the delivery of cefuroxime to ocular tissues. Among these, nanocomposite hydrogels were developed using solid lipid nanoparticles and microemulsion in combination with Pluronic F-127 as a polymeric matrix for the intravitreal delivery of cefuroxime. The results showed a low release of the entrapped drug for up to 7 days indicating potential suitability for use as POE prophylaxis [44].

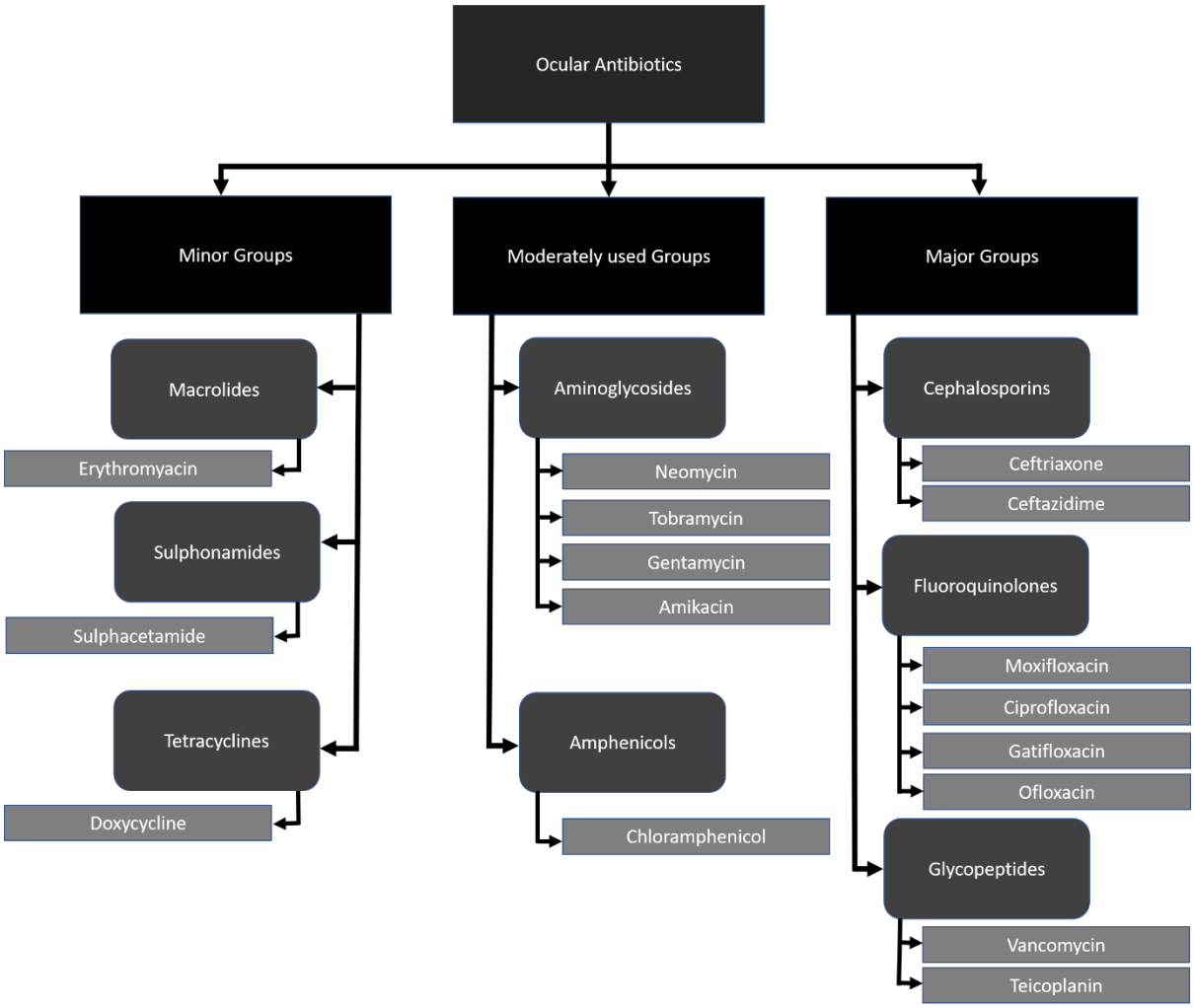

Figure 2.2. Outline of minor, moderately used, and major groups of antibiotic classes employed for POE

Moxifloxacin represents one of the well-studied fourth-generation of fluoroquinolones and it follows the working mechanism of disrupting bacterial DNA replication, transcription and repair [45]. This is achieved by the inhibition of enzymes such as topoisomerase II and

topoisomerase IV [46-49]. Furthermore, fluoroquinolones represent a mostly used class of antibiotics owing to their potency against gram-positive and gram-negative bacteria. However, there has been a recent substantial increase in fluoroquinolone resistance and this is a growing concern [50]. The hydrogel system developed as carrier of moxifloxacin make use of thermoresponsive polymers such as Pluronic F-127 conjugated with chitosan [51], poly(N-isopropylacrylamide) with poly(ethylene glycol) [52], Pluronic F-127, gellan gum and Carbopol [53, 54] to mention a few. Some of the desirable properties of the hydrogel carriers include, but not limited to, improved biocompatibility [55], enhanced mucoadhesion [37], increased potency [56, 57], prolonged release [15, 58], biodegradation [59], and bioresorption [60] which is crucial for ocular drug delivery systems.

Vancomycin has been reported to be an effective candidate of intravitreal administration and represents one of the glycopeptide antibiotics that are extensively used for POE [38, 61]. This is owing to its excellent activity against gram positive organisms (i.e.; *staphylococcus aureus*) which is achieved by prevention of peptidoglycan polymerization which in turn weakens the bacterial cell wall. However, the use of vancomycin against gram negative organisms has shown poor to no activity [62]. Furthermore, the use of vancomycin for surgical prophylaxis has been cautioned in order to prevent the spread of vancomycin resistance [63]. Hydrogels that used vancomycin for treatment of POE includes thermoresponsive polymers such as poly(ethylene glycol) diacrylate and poly(N-isopropylacrylamide) for sustained release, enhanced microbial potency, and accelerated biodegradation and resorption [17].

2.3.1.1. Approaches for Encapsulating and Delivering Ocular Antibiotics via the use of Thermoresponsive Hydrogels

In addition to thermosensitive hydrogels, several approaches have been explored for enhancement of antibiotic delivery to ocular tissues. These include drug complexation, polymer conjugation/blending, copolymerisation, and crosslinking as shown in Figure 2.3 [64]. Ocular antibiotic complexes have been reported to show significant improvements in drug retention capabilities, drug lipophilicity, and improved antimicrobial efficiency [65]. Cyclodextrins have gained popularity in ocular antibiotic therapy as solubilizers, stabilizers, and permeation enhancers [66-68]. Polymer blending has also been explored for enhanced ocular antibiotic delivery. The blending of thermoresponsive polymers with polysaccharides and cellulose derivatives has been reported to offer provision of higher gel strength and viscosity, thereby adding control over drug release [56, 64, 69]. Pluronic F-127and chitosan were blended according to the cold method to allow high percentage drug incorporation

(97.69%) [70]. Other forms of blending include varying the ratios of polymers to facilitate the thermoresponsive properties of the hydrogel [71]. In addition, varying polymer ratios aids in tuning the gelation kinetics [72].

The other form of loading antibiotics on to thermoresponsive hydrogels makes use of copolymerisation of polymers such as poly(N-isopropylacrylamide) as a thermoresponsive polymer and a functionalised chitosan to improve gel strength, increase carrier biodegradation resistance in order to prolong the release profile [73, 74], poly(acrylic acid) grafted poly(N-isopropylacrylamide) for controlled drug release [75], and the employment of thermoresponsive block copolymers (i.e. diblock, triblock, and novel pentablock) for enhanced rheological properties to promote sustained drug release with limited initial burst release [76, 77]. In addition, research is moving towards exploiting combinations of the above-mentioned approaches to get the desired properties of each. For example, thermoresponsive chitosan-B-glycerophosphate and pH responsive copolymerization of poly(N-isopropylacrylamide) and itaconic acid were blended to allow for sustained release [78].

Crosslinking of thermoresponsive polymers is another approach that has been explored for enhancement of antibiotic delivery to ocular tissues for provision of sustained release of antibiotics. Dosmar *et al*. reported a burst release of 23%, 31%, and 36% at 6, 12, and 24 hours, respectively. This was followed by a prolonged release for 2 weeks after crosslinking vancomycin loaded poly(ethylene glycol) diacrylate and poly(N- isopropylacrylamide) with *N,N,N',N'*-Tetramethylethylenediamine [17]. Other studies made use of physical crosslinking, chemical crosslinking [79, 80], dual crosslinking [22, 81] to facilitate prolonged drug release to targeted ocular tissues. For example, genipin crosslinked carboxymethyl chitosan and Pluronic F-127 showed improved drug facilitation for transcorneal penetration and prolonged the precorneal drug retention time [57]. Chitosan based thermo-gelling systems are becoming popular in ocular drug delivery. These are developed by crosslinking the aqueous solution of chitosan with polyol salts such as disodium glycerophosphates salts [82]. Incorporation of β-glycerophosphate in ocular drug delivery systems is essential for the enhancement of the systems' pH to 7.0–7.4 which is the physiological range. Furthermore, β-glycerophosphate allows for controlled gel formation when an increase in temperature is imposed and this is desirable in the development of *in situ* gelling hydrogels [83].

The stimulation of sol-gel transition of *in situ* gelling hydrogels requires appropriate amount of triggers and mechanisms for improved mechanical strength [84]. Ideally, an optimal POE treatment or prophylaxis drug delivery system will show desirable results such as but not

limited to reducing the frequency of drug administration, allowing for enhanced bioavailability and maximum drug entrapment, loading and release by overcoming the ocular barriers, and protecting the encapsulated drug for achievement of drug release in a controlled manner [34, 56]. During instillation into the eye, the *in situ* thermo-gel-forming system appears as a liquid, but swiftly converts into a viscoelastic gel after administration. As a result, the gel generated *in situ* will have a substantially longer residence time, and the encapsulated drug will be released sustainably over time. Thereby increasing bioavailability and minimizing the frequency of administration [20]. Cheng *et al*. developed a sustained levofloxacin releasing hydrogel system for post-cataract surgery infections. This hydrogel demonstrated a slow drug release profile for 7 days due to its ability to undergo a sol-gel transition at the temperature of 34.15 ± 0.17 ºC. Furthermore, this was enhanced by the rapid sol-gel transition which was reported to occur within 81.73 seconds [85]. Similarly, a sustained release was reported from a levofloxacin loaded poly(*D,L*-lactic-*co*-glycolic acid)-block-poly(ethylene glycol)-block-poly(*D,L*-lactic-*co*-glycolic acid) where a burst release of approximately 20% of levofloxacin was observed for 48 hours followed by a sustained release for one month [86].

The studies reported above highlight the efficacy of approaches such as drug complexation, polymer conjugation/blending, copolymerisation, and crosslinking in loading and releasing antibiotics. However, there is still no single antibiotic that covers efficiently all the organisms that induce postoperative endophthalmitis especially in cases where inflammation is present. Therefore, a combination of at least two drugs (i.e. an antibiotic and steroid) is thus required.

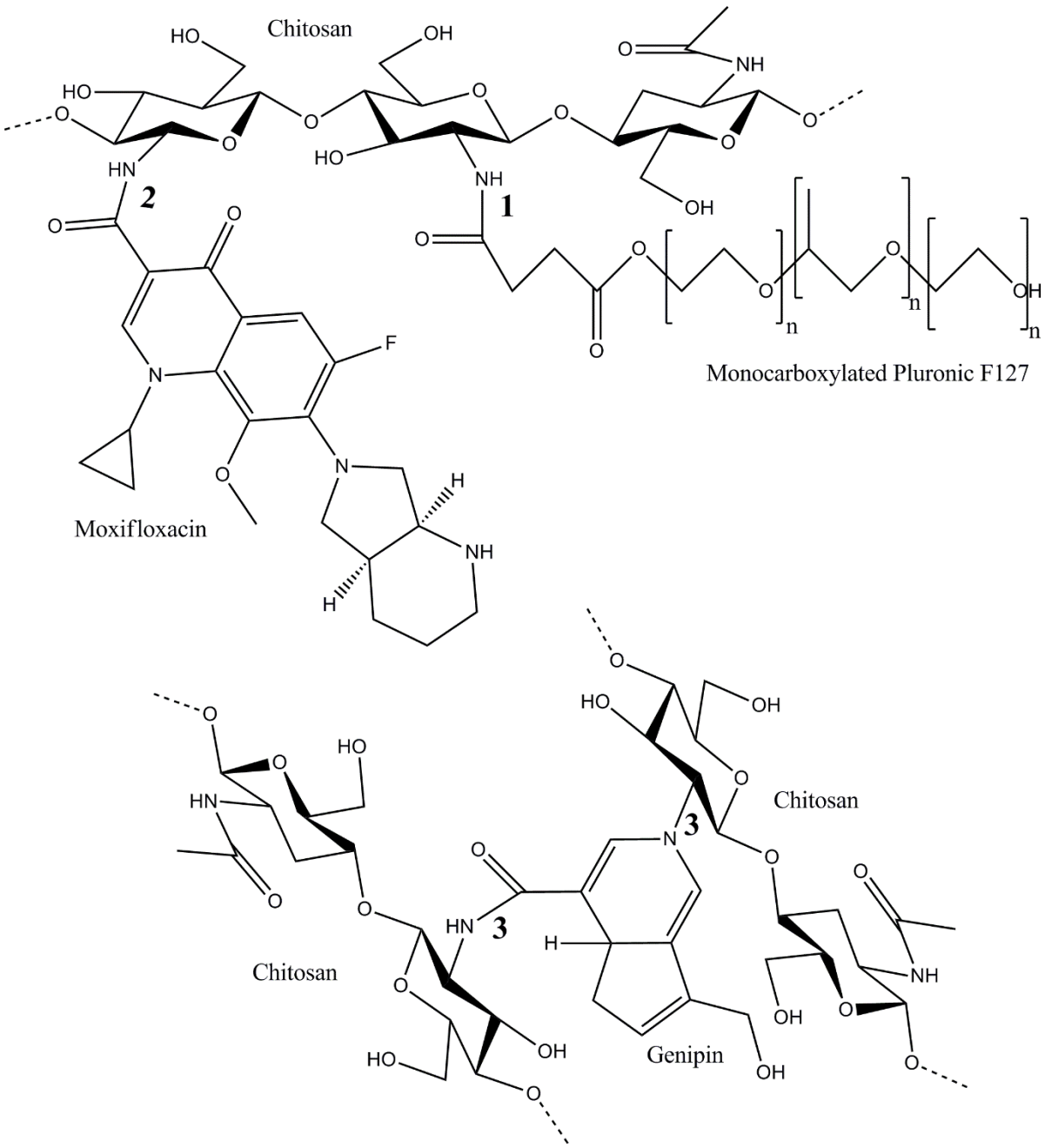

Figure 2.3. Structural representation of polymer conjugation/copolymerisation of chitosan with monocarboxylated Pluronic F-127 in EDC/NHS (1), polymer-drug complexation of chitosan-moxifloxacin complex (2), and chemical crosslinking of chitosan with genipin (3)

2.3.2. Thermoresponsive Hydrogels for Delivery of Ocular Corticosteroids

The rationale behind using steroids is the requirement of an anti-inflammatory agent in endophthalmitis. This is based on the histological results of eyes with endophthalmitis where a severe inflammation is present. Histopathologic studies on POE ocular tissue have indicated that in some cases bacterial infections may lead to infiltration of immune cells causing retinal destruction [87]. Based on this, the use of corticosteroids is to suppress the inflammation and

restore the retinal structure [88]. The most studied corticosteroids for POE include triamcinolone acetonide, prednisolone, fluorometholone, loteprednol etabonate and dexamethasone being the most understood [87, 89, 90].

Triamcinolone acetonide is an intermediate-acting potent corticosteroid that is a relatively safe and effective agent for treatment of conditions requiring long-term ocular steroid administration [91]. The duration for administering these corticosteroids for long term conditions can be up to 42 days which can be achieved by using polymeric conjugations such as poly-lactic-co-glycolic acid [92]. However, development of formulations containing triamcinolone acetonide poses a challenging task owing to its poor solubility and permeability. To circumvent this problem, solid lipid nanoparticles and *in situ* hydrogels can be employed to enhance permeation of triamcinolone acetonide across ocular tissues. The use of solid lipid nanoparticles has been reported to aid transcorneal permeability while the *in situ* hydrogel assisted with the retention of drug resident time thereby prolonging release [93].

Difluprednate is a novel derivative of prednisolone which is a synthetic glucocorticoid derived from cortisol [94, 95]. Difluprednate is used for POE due to its high potency, favourable safety profile, and the ability to reduce postoperative pain [96]. Substitution of the 21-hydroxyl group on the difluprednate molecule with acetate has been reported to enhance corneal penetration [97]. Furthermore, previous studies have reported that difluprednate has excellent efficacy in resolving postoperative inflammation [98, 99]. However, the use of this drug has been associated with increased incidence of intraocular pressure [100]. Other corticosteroids that have also been explored for POE include prednisolone acetate and loteprednol etabonate which is an ester-based corticosteroid used for postoperative ocular pain and inflammation [101]. Prednisolone acetate loaded chitosan-sodium deoxycholate nanoparticles showed enhanced bioavailability and enhanced penetration to ocular tissues [95].

Fluorometholone is one of the many corticosteroids that have been studied for possible use in POE. This is due to its high anti-inflammatory potency and low risk of increasing intraocular pressure as a result of the fluorometholone to dihydrofluorometholone transition during corneal penetration [102]. However, fluorometholone has been reported to have a low corneal penetration which may pose a challenge of ineffective therapeutic levels [103]. Strategies to improve this include among others incorporating fluorometholone into polymeric nanoparticles for enhanced protection from the enzymatic degradation by the tear film [104]. The hydrogel nanosystems developed as carrier of fluorometholone make use of thermoresponsive polymers such as Pluronic F-127 incorporating poly (lactic-co-glycolic acid) nanoparticles for increased bioavailability [103].

Dexamethasone is a long-acting glucocorticoid with a high ocular potency and efficacy [105]. Dexamethasone, the most commonly used ocular corticosteroid, has been demonstrated in multiple studies to reduce discomfort and inflammation following cataract surgery at a low concentration (0.1% $^{w}/_{v}$) [106, 107]. Furthermore, its anti-inflammatory activity has been reported to be more potent in comparison with that of prednisone and prednisolone [36]. Despite the fact that dexamethasone has been shown to reduce ocular inflammation, if it is not taken properly, the impact may not be fully realized [108, 109]. Although controversial, the use of intravitreal dexamethasone is widely accepted as studies report that it shows no major safety concerns when used for acute POE prophylaxis [108]. Some of the thermoresponsive hydrogels used to incorporate dexamethasone include but not limited to Pluronic F-127 conjugated with Pluronic F-68 for enhanced precorneal retention [110], and crosslinked copolymers of poly(N-isopropylacrylamide-co-methacrylate-co-vinyl pyrrolidone) for improved ocular bioavailability [111].

Literature reports state that corticosteroids have a potentially beneficial role in POE. However, there are varying reports on the acceptable route of administration, with most controversies persisting in regard to the intravitreal steroid therapy [88]. Several concerns have been raised with regards to the use of intravitreal corticosteroids in POE. These include concerns of retinal toxicity, possible effect on concomitant antibiotics pharmacokinetics, and controversies around the use in fungal endophthalmitis [87, 112]. Therefore, potential approaches to the treatment and prophylaxis of POE should allow for sufficient anti-inflammatory response to clear bacteria while minimizing retinal toxicity. Among these, punctal plug depot systems have been proposed as alternatives for ocular delivery of corticosteroids due to their ability to provide drug delivery over a desired period of time with the added ability of tapering concentrations [113]. To elucidate the plug system approach, dexamethasone was loaded into polyethylene glycol intracanalicular plug and the system exhibited sustained drug release of over 30 days for treatment of ocular inflammation associated with ocular surgery [114].

Studies based on animal models of endophthalmitis have demonstrated clinical improvement with intravitreal steroids added to the antibiotic regimen [87]. Literature reports indicates that repeated intraocular injections may be the cause of exacerbating the drawbacks associated with intravitreal corticosteroids [115, 116]. Therefore, the goal for corticosteroid delivery systems is to prolong vitreal residence time to avoid multiple injections. A previous study indicated that ocular drug release from hydrogels is limited to the timeframe of hours to days [71]. However, research advancements have introduced nanosystems such as solid lipid nanoparticles, liposomes, dendrimers, and micelles which are capable of providing longer drug

release profiles thereby increasing corticosteroid solubility in aqueous solution [90, 117]. The incorporation of these systems into thermoresponsive hydrogels lead to fabrication of easily administered, and biocompatible systems that offer clinically beneficial prolonged drug release for days to months [52, 118]. Furthermore, studies have demonstrated that nanostructured lipid carriers can be used to encapsulate and deliver poorly soluble drugs while enabling greater drug permeation into tissues via lipid membrane fusion [110, 119].

To elucidate the use of nanolipid systems approach in delivery of corticosteroids, a composite hybrid thermosensitive *in situ* gelling hydrogel was formulated by employing hydroxypropyltrimethyl ammonium chloride chitosan/ β-glycerophosphate loaded with dexamethasone-nanostructured lipid carrier for ocular delivery to enhance the apparent solubility of dexamethasone [13]. This drug delivery system was reported to undergo a sol-gel transition at the temperature of 35 ºC and this occurred within a short period (3 minutes) [13]. In addition, the entrapped dexamethasone release followed a sustained drug release pattern for a period of 3 days [13]. This sustained release could be attributed to the diffusion of the drug molecules through the added barrier as a result of entrapment in the nanostructured lipid carrier thereby slowing down the release. Similarly, Mo and colleagues explored the idea of incorporating a secondary drug carrier by developing a dexamethasone loaded nanostructured lipid carrier in combination with thermosensitive Pluronic F-127 and F-68 to improve the drug bioavailability and therapeutic efficacy [110]. These findings substantiate the nanostructured lipid carrier's ability to penetrate across ocular tissues thereby increasing mucoadhesivity.

Pluronic F-127 and Pluronic F-68 are widely used in the development of thermosensitive *in situ* gels for delivery of corticosteroids. This is because they can easily be adjusted to formulate long acting system with desirable gelation properties [18]. These gelation properties are important particularly in ocular drug delivery systems [120]. For instance, a slow gelation time may lead to difficulties for the system to form a gel resulting in a rapid flow or elimination, whereas, a fast gelation time may lead to a non-flowable gel which will result in difficulties during administration [18]. Accordingly, the decrease in Pluronic F-127 to concentrations below 16% ($^{w}/_{w}$) resulted in an increased sol-gel transition temperature whereas concentrations above 20% ($^{w}/_{w}$) resulted in the sol-gel transition temperature lower than room temperature [110, 121]. Therefore, the use of concentration of 18% ($^{w}/_{w}$) for Pluronic F-127 was selected as the most suitable and resulted in the sol-gel transition at a temperature of 34.8 $^{\circ}$C within 27.3 seconds [110]. As a result of the relatively fast gelation rate, a sustained dexamethasone release of 80% was observed after 35 hours [110]. In order to preserve the

administered drugs, a considerably rapid gelation time is of critical importance in ocular *in situ* systems. This is to avoid drug elimination by the eye mechanisms such as the blinking reflex, rapid tear turnover and nasolacrimal drainage effect thereby improving ocular drug retention [22].

2.3.3 Thermoresponsive Hydrogels for Delivery of Ocular Dual Drugs

In comparison to extemporaneous combinations, fixed drug combinations have been reported to be superior for POE [122]. A literature report indicated that intravitreal corticosteroid therapy used for bacterial endophthalmitis should be concurrently injected with antibiotics [8]. Chemical-pharmaceutical research, as well as preclinical and clinical trials, all attest to this [36, 94, 123]. The advantages of fixed medication combinations are based on non-clinical toxicity profiles and safety margins for human usage, as well as efficacy and safety in people. Furthermore, because the strain of delivering many drugs is decreased, fixed drug combinations lower the risk of incorrect dosage and enhance patient compliance. Extemporaneous combinations, on the other hand, have been observed to be primarily based on practical use, and their risk to patients may be underestimated [122].

Some of the fixed antibiotic/steroid combinations researched for prophylaxis of POE include ciprofloxacin HCL/dexamethasone, Ciprofloxacin/ hydrocortisone, gentamycin sulfate/ prednisolone, triamcinolone/moxifloxacin/vancomycin [124], neomycin/ polymyxin B/dexamethasone, neomycin/ polymyxin B/hydrocortisone, tobramycin/dexamethasone [125], tobramycin/loteprednol etabonate, gatifloxacin/prednisolone acetate [126], and levofloxacin/dexamethasone sodium phosphate [125, 127]. The use of these drug combinations for POE prophylaxis has been shown in the literature to reduce costs, reduce dosage, and increase patient compliance in the early post-operative period [125]. In addition, the fixed antibiotic/steroid combinations has been reported to not cause increased intraocular pressure in about 97% of cases in the post-operative period [94].

Thermosensitive chitosan-based hydrogels have been extensively investigated for ocular use. Amongst these, thermosensitive chitosan/gelatin/glycerol 2-phosphate disodium salt hydrate hydrogel was employed to incorporate prednisolone acetate and levofloxacin for the simultaneous achievement of long-term release for optimal anti-inflammatory and anti-bacterial properties. To ensure further optimal results, PLGA was included as a nanocarrier to aid in the loading and delivery of the lipophilic levofloxacin and further promote extended release of incorporated drugs thereby improving ocular bioavailability [128]. Another chitosan based hydrogel system was fabricated for dual incorporation of dexamethasone and

levofloxacin by employing glycol chitosan/oxidized hyaluronic acid hydrogel film for extended precorneal retention [14]. In addition, the functionalized glycol chitosan formed an ester bond linkage with dexamethasone which increased the solubility of dexamethasone thus increasing bioavailability [14, 129].

Table 2.3. Thermoresponsive hydrogels for delivery of ocular drugs for POE prophylaxis

Thermo-responsive hydrogel Composition	Drugs		Gelation tempera-ture	Release profile	Route	Ref.
	Antibiotic	Corticosteroid				
chitosan/gelatin/glycerol 2-phosphate disodium salt hydrate	levofloxacin	-	34.15 ± 0.17°C	Sustained release for 7 days	Topical eye drop	[85]
poly (ethylene glycol) diacrylate/poly(N-isopropylacrylamide)	vancomycin	-	32 °C	Sustained release for up to 21 days	Subconjunctival injection	[17]
Chitosan/β-glycerophosphate	Moxifloxacin, gentamicin	-	29 to 37 °C	Controlled release for 4 hours	Topical eye drops	[130]
Pluronic F-127, Pluronic F-68	Moxifloxacin	Dexamethasone	> 32 °C	Sustained moxifloxacin, dexamethasone, and genistein release for 10, 30, and 40 days, respectively.	Intracameral injection	[18]
Pluronic F-127, Hydroxyl propyl methyl cellulose K4M	Tobramycin sulphate	Dexamethasone sodium phosphate	37.30 °C	Sustained release for over 9 hours	Topical eye drops	[_EN REF_ 143131]
Pluronic F-127	-	Fluorometholone	34 ± 0.5 °C	Sustained release for 48 hours	Topical eye drops	[111]
chitosan/gelatin/glycerol 2-phosphate disodium salt hydrate	Levofloxacin	prednisolone acetate	34.3 ± 0.3 °C	Sustained release for up to 7 days	Topical eye drops	[128]
Pluronic F-127	Cefuroxime	-	30.4 °C	Sustained release for up to 1 week	Intravitreal	[44]

	injection

2.4. Prospective Trends

2.4.1. Delivery of Hydrophobic Drugs

There is still a challenge in delivering hydrophobic drugs (i.e.; dexamethasone, prednisolone acetate, difluprednate, loteprednol etabonate) using hydrogels. This is due to the inherent incompatibility of the hydrophilic hydrogel network with the drug. Extensive research has been conducted on how to successfully load the hydrophobic drugs into the gel matrix and once loaded, there is still limitations in effectively releasing the drug into the aqueous environment [132]. Recent work suggests that the strategies for improving ocular penetration of ocular drugs have included the use of mucoadhesive formulations (i.e., polycarbophil-containing gels) [94], the use of cyclodextrins to increase the water solubility of lipophilic drugs [133] and also the employment of nanostructured lipid carriers. Advancement in smart multitargeting drug delivery by incorporating nanocarriers are gaining considerable attraction as a result of their potential for a more efficient drug release [134]. A study by Yan *et al*. demonstrated the use of different approaches in loading multiple drugs into thermoresponsive hydrogels to control the reals in an attempt to treat POE. Genistein was entrapped in nanostructured lipid carriers and dexamethasone was combined with calcium chloride, while moxifloxacin was added directly into the hydrogel [18]. *In vitro* results showed no burst release for all drugs. However, moxifloxacin produced a shorter sustained release occurring within 10 days [18]. This was attributed to the direct encapsulation of moxifloxacin into the hydrogel. Dexamethasone on the other hand had a 50% release from the hydrogel in the first week, followed by a sustained release until day 30 [18]. This is as a result of the supramolecular hydrogel formed between dexamethasone and the added calcium ions. Furthermore, the use of calcium chloride increased the crosslinking density, adhesiveness, and the stringiness of the hydrogel which further added to the controllable release velocity. Genistein showed a prolonged release profile for at least 2 months with no burst release. This was reported to be due to a double-controlled release process caused by the incorporation of the genistein loaded nanostructured lipid carrier into the hydrogel [18]. The study above provides insights on different approaches can be employed to load drugs which can improve properties of the hydrogels to obtain desirable outcomes such as prolonged release, optimal gelling temperatures and combating secondary infections in POE.

2.4.2. Intraocular Lens Therapy

Recent research advancements are introducing the use of intraocular lens platforms for controlled release of ocular drugs. The desirable features of these drug delivery platforms include the avoidance of frequent instillation, increase in ocular residence time which will in turn minimize drug drainage, and ensure better compliance [135]. Intraocular lens have been used as lens replacement for natural lens affected by cataracts and it has been established that they are well tolerated by patients therefore, they are ideal devices to be used as drug carriers for treatment or prophylaxis of POE [136]. Several studies have explored the possibility of using intraocular lens devices as drug delivery systems for sustained drug release [16, 136, 137]. However, limited research has been conducted on the possibility of using intraocular lens devices for simultaneous delivery of an antibiotic and a corticosteroid POE prophylaxis [137]. One of the studies that explored the possibility of loading multiple drugs for possible use as prophylaxis of acute post-cataract surgery endophthalmitis made use of CI26Y (80–90% of 2-hydroxyethyl methacrylate and 10–20% of methyl methacrylate) to incorporate moxifloxacin with ketorolac and moxifloxacin with diclofenac for sustained simultaneous drug release [16]. Furthermore, the concept of using intraocular lens devices for POE has further gained considerable attention due to the fact that the lens device is implantation and remains in the eye post-surgery as a replacement for the natural lens [138]. Therefore, this makes it possible to combine cataract surgery with postoperative care in a single procedure.

2.5. Concluding Remarks

There has been extensive development of novel *in situ* gelling thermosensitive hydrogels systems carrying antibiotics and corticosteroids for POE prophylaxis. Recent research studies demonstrated that the use of *in situ* gelling thermosensitive hydrogels systems significantly enhance the therapeutic efficacy and bioavailability of ocular antibiotics and corticosteroids, compared to the established traditional ocular drug delivery strategies. Many attempts have been made to develop ocular drug delivery systems to replace post-operative eye drop. This is essential in improving post-operative patient compliance. Furthermore, the ability to reduce the need for eye drops after ocular surgeries is a big step towards therapeutic success, not only for adults, but also for children for whom post-operative eye drop use may prove more challenging to implement and achieve full compliance. Intraocular injections (intracameral or intravitreal) of antibiotics and corticosteroids is recommended by the European Society of Cataract and Refractive Surgeons (ESCRS) [139]. However, there is no global consensus with regards to this procedure due to factors such as lack of a commercially-available preparations and legal barriers. There is limited research into novel formulations containing both antibiotics and corticosteroids loaded into thermoresponsive hydrogels. The future success of antibiotics

and corticosteroids for POE prophylaxis could highly benefit from innovative material development such as fabrication of mucoadhesive formulations, dual drug loaded thermoresponsive hydrogels, and nanostructured lipid carriers for improving ocular penetration. Other areas to be explored include employing intraocular lens devices as carriers for antibiotics and corticosteroids which can offer sustained simultaneous drug release for possible use as prophylaxis of acute post-cataract surgery endophthalmitis.

2.6. References

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CHAPTER 3

Fabrication of a dual drug-loaded thermoresponsive chitosan-gelatin hydrogel for potential treatment of post-cataract surgery infection and inflammation

3.1. Introduction

There are several problems associated with post-surgical treatment of ocular conditions such as cataract. Amongst these concerns, are the development of secondary infections and inflammation which are extensively controlled by a routine administration of an antibiotic agent as part of a prophylactic post-operative regimen in conjunction with corticosteroids for the management of post-operative inflammation [1]. Reports have indicated that poor post-operative care could lead to the development of further complication such as increased intraocular pressure, secondary glaucoma, posterior capsular opacification, irreversible blindness and cystoid macular oedema which could negatively impacting the healing outcome thereby delaying recovery [2].

Several drugs have been widely researched for their use in the treatment of the above-mentioned complications. The used drugs include but not limited to loteprednol etabonate, difluprednate, fluorometholone, dexamethasone, or prednisolone as anti-inflammatory agents [3], and fluoroquinolones, aminoglycosides, cephalosporins, and chloramphenicol as antibiotic classes [4]. Despite the wide selection of drug molecules to choose from, ocular drug administration is still faced with several challenges attributed to the anatomy and physiology of the eye such as ease of drug removal by lacrimation, nasolacrimal drainage, tear dilution and tear turnover [5], limited bioavailability due to less drug available to exert desired therapeutic effects [6], or toxicity [7].

This led to the encapsulation of these drug molecules in drug delivery systems such as contact lenses [8], eye drops [9], ocular inserts, implants [10], and also hydrogels [11] with eye drops being the conventional approach in treatment of ocular post-surgical intervention. This is due to their ease of use and also their less invasive nature. However, this drug delivery approach has raised several concerns in terms of poor patient compliance. This poor patient compliance has been attributed to patient factors such as improper dose administration techniques such as blinking prematurely, missing the eye, instilling incorrect amounts, abrupt discontinuation and contaminating the bottle tip by touching the ocular surface or failure to wash hands before administration [2]. Another important factor is the frequent application necessitated by the use

of eye drops with which patients often fail to comply with, thus affecting the bioavailability. Accordingly, reports have strongly indicated that approximately 5% of the applied dose of eye drop preparations reaches the intraocular tissues, which adversely affects drug efficacy [9, 12, 13].

Other ocular devices such as ocular inserts, contact lenses and implants are also associated with drawbacks such as ocular tissue irritations, invasive procedures, and biodegradability limitations [14, 15]. It is evident that advances have been made towards improving ocular drug delivery systems. However, the defence mechanism of the eye constantly and continuously works against these systems as they are foreign bodies. An alternative way forward that could potentially yield better results would be to develop a delivery system that will proactively release drugs in response to a specific stimulus at the target site [16]. To this end, development of stimuli-responsive and biocompatible hydrogel systems has received much attention due to their effective drug delivery properties [11].

Thermoresponsive hydrogels are amongst the widely investigated drug delivery biomaterials for post-cataract surgery care. Selection of thermoresponsive polymers plays a crucial role in screening suitable drug delivery biomaterials. This includes the capability of the polymers to retain their thermoresponsive nature after forming a hydrogel and also be able to interact with drug molecules for a sustained release [17, 18]. Amongst these thermoresponsive polymers is β-Glycerophosphate (BGP) crosslinked chitosan which assumes its thermoresponsive behaviour after crosslinking [19]. Chitosan (CS) is natural polymer and a suitable candidate for ocular formulation due to its desirable biological properties such as less to no toxicity towards ocular tissues [20], antimicrobial properties [21], gel forming capabilities [22] and also the ability to facilitate wound healing [19]. BGP is nontoxic ionic crosslinker that is mainly used as a catalyst for a swift sol-gel transition in CS solutions at physiological conditions i.e.; pH and temperature [23]. Reports have indicated an increase in pH into the physiological range of 7.0-7.4 upon the addition of BGP to CS solutions and this has been attributed to the neutralizing effect induced by the presence of phosphate groups in BGP [23, 24].

Formulating CS and gelatin-based hydrogels has been reported to form mucoadhesive hydrogels with desirable mechanical properties along with high cytocompatibility and efficient drug release [25]. Gelatin is also a naturally derived polymer with highly biocompatibility properties. A chitosan and gelatin blend possess multiple -NH_2 functional groups that enable optimization by form of crosslinking to facilitate a more structurally rigid hydrogel with feasible rheological properties [25].

Genipin (GEN) is a nontoxic natural derived chemical crosslinker with great affinity for polymers and proteins containing primary amine groups and capable of producing highly rigid hydrogel structures [25]. GEN possess groups such as -OH and $-COO^-$ which could spontaneously react with the $-NH_2$ groups on CS-GEL blends. Compared to other crosslinkers, genipin is reported to demonstrate higher biocompatibility and lower cytotoxicity. Crosslinking modified chitosan and gelatin (GEL) with genipin has been reported to improve drug release performance of hydrogels [24, 26]. Enhanced bioavailability and sustained release of sodium fluorescein was reported to be facilitated by crosslinking CS/gel-based hydrogels [24]. Herein, polymers such as chitosan and gelatin were crosslinked with β-glycerophosphate and genipin to develop an *in situ* forming hydrogel capable of dual release of dexamethasone sodium phosphate (DSP) and moxifloxacin HCL (MOX) for post-cataract surgical treatment. The dual drug encapsulation is a post-surgical intervention to target both inflammation and development of secondary infection that could occur in the anterior segment of the eye. The application of naturally derived polymers and crosslinkers would aid cytocompatibility and biocompatibility in terms of presenting optimal mechanical properties for use as an *in situ* forming injectable thermoresponsive hydrogel.

3.2. Materials and Methods

3.2.1. Materials

Low molecular weight chitosan, Gelatin (PH EUR), β-Glycerophosphate disodium salt hydrate, genipin, and phosphate-buffered saline (PBS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Simulated tear fluid (STF) comprised 6.8 g NaCl, 2.2 g $NaHCO_3$, 0.08 g $CaCl_2 \cdot 2H_2O$, 1.4 g KCl, and deionised water to 100 mL. NaOH and HCl were used to adjust pH to 7. All materials employed were of analytical grade and used without further purification.

3.2.2. Fabrication of thermoresponsive thermo-ocugel hydrogels

CSBGP was prepared by dissolving 2 g CS in 50 mL 1% aqueous acetic acid solution. A 3 % ($^w/_v$) BGP solution was prepared and added drop wise into CS solution using a 0.22 µm syringe filter. The CS-BGP-GEL-MOX-DSP-GEN (thermo-ocugel) was formulated via dissolving 0.1 g GEL in 10 mL deionised water under constant stirring at 50 ^oC and cooled for further use. MOX and DSP were added to the gelatin solution to final concentrations of 0.5 and 0.1% ($^w/_v$), respectively. The GELMOXDSP solution was kept at room temperature (25 ^oC) under constant stirring for 1 hour before they were mixed with CSGBP to form the CSBGPGELMOXDSP. The reaction was allowed to proceed for 2 hours before genipin was added drop wise under constant stirring for crosslinking to a final concentration of 1 % ($^v/_v$). The reaction mixture was

incubated at 37 °C for 24 hours at a speed of 50 rpm in the orbital shaking incubator (LM-530-2, MRC Laboratory Instruments Ltd. Hahistadrut, Holon, Israel) to allow for gel formation. The following formulations were prepared for characterisation; CSBGP (F1), CSBGPGEL (F2), CSBGPGELGEN (F3), and CSBGPGELMOXDSPGEN (thermo-ocugel).

3.2.3. Evaluation of molecular and chemical characteristics of biomaterials via FTIR

The FTIR spectra analysis was carried out for the determination of specific interactions between the employed biomaterials and crosslinkers in forming hydrogels. FTIR parameters were set as follows; 20 scans and a wavenumber range of 4000–650 cm^{-1} using a PerkinElmer Spectrum 2000 ATR-FTIR (PerkinElmer 100, Llantrisant, Wales, UK) spectrometer fitted with a single-reflection diamond MIRTGS detector. The following samples were evaluated; CS, BGP, GEL, MOX, DSP, GEN, F1, F2, F3, and the thermo-ocugel.

3.2.4. Evaluation of the diffraction pattern of lyophilized hydrogels via XRD

The biomaterials together with lyophilized hydrogel scaffold diffraction patterns were analysed by X- ray diffraction to characterize the types of phases present. X-ray diffraction spectra were recorded on a benchtop MiniFlex 600 (Rigaku, Japan) diffractometer using CuK_{α} radiation at 40 kV and 15 mA. The 2θ scan range was selected between 3–90 degrees at a scan rate of 10 degrees/minute. This diffraction analyses allowed for the observation of the degree of crystallinity and phase changes on hydrogels.

3.2.5. Thermostability assessment of biomaterials and hydrogels

The thermophysical properties of the fabricated hydrogels and biomaterials were determined using a differential scanning calorimeter (DSC) (Mettler Toledo, DSC, STARe System, Swchwerzenback, ZH, Switzerland). Lyophilized hydrogels and biomaterial samples of 3 to 10 mg were sealed in aluminium crucibles and heated over a temperature range of 0 to 400 °C at a heating rate of 10 °C/min under a N_2 atmosphere. DSC curves were plotted as heat flow against temperature. The temperature range within which the formulations degraded were determined using a thermo-gravimetric analyser (TGA) (PerkinElmer, TGA 4000, Llantrisant, Wales, UK). Samples were heated at a rate of 10 °C/min from 30 to 900 °C under continuous nitrogen purging. Thermograms were generated as percentage weight vs. temperature.

3.2.6. Assessment of mechanical properties of hydrogels

The hydrogels were evaluated using the ElastoSens™ Bio^2 (Rheolution instruments, Canada). The thermoresponsive nature of the hydrogels was assessed by following the storage modulus (G') over increasing temperature from 15 to 45 °C. The interactions between employed polymers, crosslinkers and drug molecules was also investigated by following the storage modulus of the hydrogels over time. The investigation looked into the changes in rigidity (G') of the hydrogels associated with interactions between the biomaterials. The gelling, ionic and chemical interaction, and viscoelastic (G' and G*) properties of the hydrogels was determined over time. Further rheological analysis of the formulated hydrogels was carried out using a Haake Modular Advanced Rheometer System (II) Modular Advanced Rheometer system (Thermo Fisher Scientific, Johannesburg, South Africa), with a cone plate geometry (Rotor C35/ 1, D = mm, 1 Titan) that has a gap measurement of 0.050 mm. The F1, F2, F3 and thermo-ocugel were prepared and stored at 37 °C for 24 h before determining their rheological properties. The following types of rheological experiments were conducted at: 37 °C for oscillation frequency sweep and stress sweep and 15-45 °C for temperature sweep respectively.

3.2.7. Analysis of drug loading and release from the thermo-ocugel

The thermo-ocugel was stirred in STF (pH 7.4) to allow for complete release of both DSP and MOX. The drug loading and encapsulation efficiency was determined via the use of a standard curve of the following concentrations: 0, 5, 10, 15, 20, and 25 µg/mL standard solutions of DSP and MOX in STF at wavelength 242 and 288nm, respectively. This was undertaken using UV-Vis spectrophotometry (Lambda 25 UV/Vis spectrophotometer, PerkinElmer, MA, USA). Total drug to polymer ratio were calculated in order to get the expected drug amount in a given weight of the thermo-ocugel. The expected drug mass would give insight on the encapsulation efficiency and loading of the drug by using the equations below [27]:

$$\boldsymbol{Drug\ loading}\ (\%) = \left(\frac{\boldsymbol{Mi}}{\boldsymbol{Mt}}\right)\boldsymbol{X\ 100} \qquad \textbf{[}\text{Equation 3. 1]}$$

Where *Mi* is the amount (mass) of drug obtained from the standard curve after complete release and *Mt* is the total given mass of the thermo-ocugel.

The release study was carried out by placing the formulated thermo-ocugel (5mL) in a SnakeSkin™ dialysis tubing (flat width, diameter: 35 mm, MWCO: 3.5 K, Thermo Fisher Scientific, Johannesburg, South Africa) and was exposed to physiological conditions following immersion in 100 mL STF (pH 7.4). The samples were placed in an orbital shaking incubator

(LM-530-2, MRC Laboratory Instruments Ltd. Hahistadrut, Holon, Israel) set at 37 °C with a speed of 50 rpm. Two millilitre (2mL) sample withdrawals were undertaken at predetermined time intervals (1, 2, 4, 8, 12, 24, 48, 72, 168, 336, 504, and 672 hours) followed by replacements of the same volume with fresh STF solution to maintain sink condition. Drug quantification was determined by employing the UV-Vis spectrophotometry (Lambda 25 UV/Vis spectrophotometer, PerkinElmer, MA, USA) at respective wavenumbers for each drug (DSP = 242 nm and MOX = 288 nm). All analyses were conducted in triplicate (n=3). The percentage drug release (*pt*) was obtained by comparing the percentage mass fraction of drug release at time (t) to the amount of the drug that was completely release from the thermo-ocugel (*Mi*) (Eq. 3.2). The percentage cumulative drug release was determined using Eq. 3.3 as previously reported [27].

$$\boldsymbol{pt}\ \% = \frac{\boldsymbol{Mr}}{\boldsymbol{Mi}}\ \boldsymbol{X}\ \mathbf{100} \qquad \text{[Equation 3. 2]}$$

$$\boldsymbol{Cumulative\ release}\ \% = \left(\frac{\boldsymbol{sample\ volume\ withdrawn}}{\boldsymbol{bath\ volume}}\right)\boldsymbol{X}\ \boldsymbol{p(t-1)} + \boldsymbol{pt} \qquad \text{[Equation 3. 3]}$$

Where *Mr* is the amount of drug released at the predetermined time *t*, *pt* is the percentage release at time *t* and *p(t-1)* is the percentage released before time *t*.

3.2.8. Evaluation of the surface morphology of the fabricated hydrogels

Scanning electron microscopy (SEM) (Phenom™ Scanning Electron Microscope, FEI Company, OR, USA) and energy dispersive spectroscopy (EDS) (FEI Company, OR, USA) with an accelerating voltage from 10 to 15 kV was employed for the analysis of the surface morphology of the designed formulations. The energy dispersive spectroscopy analyses were performed in an integrated system. Before analysis, all formulations were mounted on aluminium stubs using a carbon adhesive tape and coated with carbon/gold palladium in a 2:1 ratio.

3.2.9. Fluid uptake and erosion of the thermo-ocugel

Erosion and swelling analysis of the thermo-ocugel were performed under simulated tear fluid (STF). The orbital shaking incubator (LM-530-2, MRC Laboratory Instruments Ltd. Hahistadrut, Holon, Israel) was maintained at 37 °C with a speed of 50 rpm. All analysis were conducted in triplicate (n=3) where 2 mL of the formulated thermo-ocugel was added to vials and allowed to reach the gelation state at 37 °C for a period of 24 hrs. The hydrogel samples were then immersed into 5 mL STF vials. For swelling analysis, the hydrated mass of the

thermo-ocugel samples was taken at predetermined time intervals for the determination of the degree of fluid uptake over time. The degree of erosion was measured on day 1, 2, 3, 5, 7, 14, 21 and 28. In each of these days, the dried mass was measured for the determination of change in mass. The wet samples were lyophilized to ensure complete removal of residual moisture before being weighed to determine the eroded mass. Swelling was measured using Eq. 3.4. and the mass loss of the thermo-ocugel after erosion was calculated using Eq. 3.5 [28].

$$\boldsymbol{Swelling}\ (\%) = \frac{(\boldsymbol{Wh}-\boldsymbol{Wi})}{\boldsymbol{Wi}}\ \boldsymbol{X\ 100} \qquad \text{[Equation 3. 4]}$$

$$\boldsymbol{Erosion}\ (\%) = \frac{(\boldsymbol{Wi}-\boldsymbol{Wf})}{(\boldsymbol{Wi})}\ \boldsymbol{X\ 100} \qquad \text{[Equation 3. 5]}$$

Where *Wh* is the mass after hydration, *Wi* is the initial weight and *Wf* is the lyophilised thermo-ocugel.

3.2.10. Kinetic analysis of drug release from the thermo-ocugel

Kinetic models were used to describe the release kinetics of the thermo-ocugel. The zero-order model (Equation 3.6), first-order model (Equation 3.7), Higuchi model (Equation 3.8), Korsmeyer-Peppas model (Equation 3.9) and Hixson-Crowell cube root law (Equation 3.10) were used to analyse the *in vitro* release data of MOX and DSP from the thermo-ocugel [29].

$$C = k_0 t \qquad \text{[Equation 3.6]}$$

Where k_0 is the zero-order rate constant and *t* is the time.

$$Log\ C = Log\ C_0 - \frac{k_t}{2.303} \qquad \text{[Equation 3.7]}$$

Where C_0 is the initial drug concentration and k_t is the first order constant.

$$Q = Kt^{1/2} \qquad \text{[Equation 3.8]}$$

Where *K* is the rate constant.

$$Q_0^{1/3} - Q_t^{\frac{1}{3}} = K_{HC} t \qquad \text{[Equation 3.9]}$$

Where Q_t is the amount of drug released at predetermined time intervals (*t*), Q_0 is the initial thermo-ocugel drug content and K_{HC} is the Hixson-Crowell rate constant.

$\frac{M_t}{M_\infty} = Kt^n$ [Equation 3.10]

Where M_t/M_∞ represents the fraction of drug released at predetermined time (t), K is the rate constant and n is the release exponent.

3.3. Results and Discussion

3.3.1. Fabrication of a thermoresponsive hydrogel

The current study employed BGP crosslinked chitosan to induce thermo-sensitivity. The structurally modified chitosan was allowed to interact with gelatin followed by incorporation of the drugs before crosslinking with genipin. Low percentages of chitosan (2%), BGP (3%), Gelatin (0.5%), and genipin (1%) were used to allow for ease of mixing. The idea behind the use of natural polymers and crosslinkers was to aid the biocompatibility of the hydrogel. The use of a chemical crosslinking approach (genipin) was to allow the viscous mixture to mix well and only form a gel *in situ* (Figure 3.1). The ability of the hydrogel to form a gel *in situ* would allow for injection of the hydrogel in the anterior segment of the eye and the injected hydrogel will transition into a gel once it is in the eye (37 °C). Gelling of the hydrogel in the eye would prevent burst release and drug elimination in the eye.

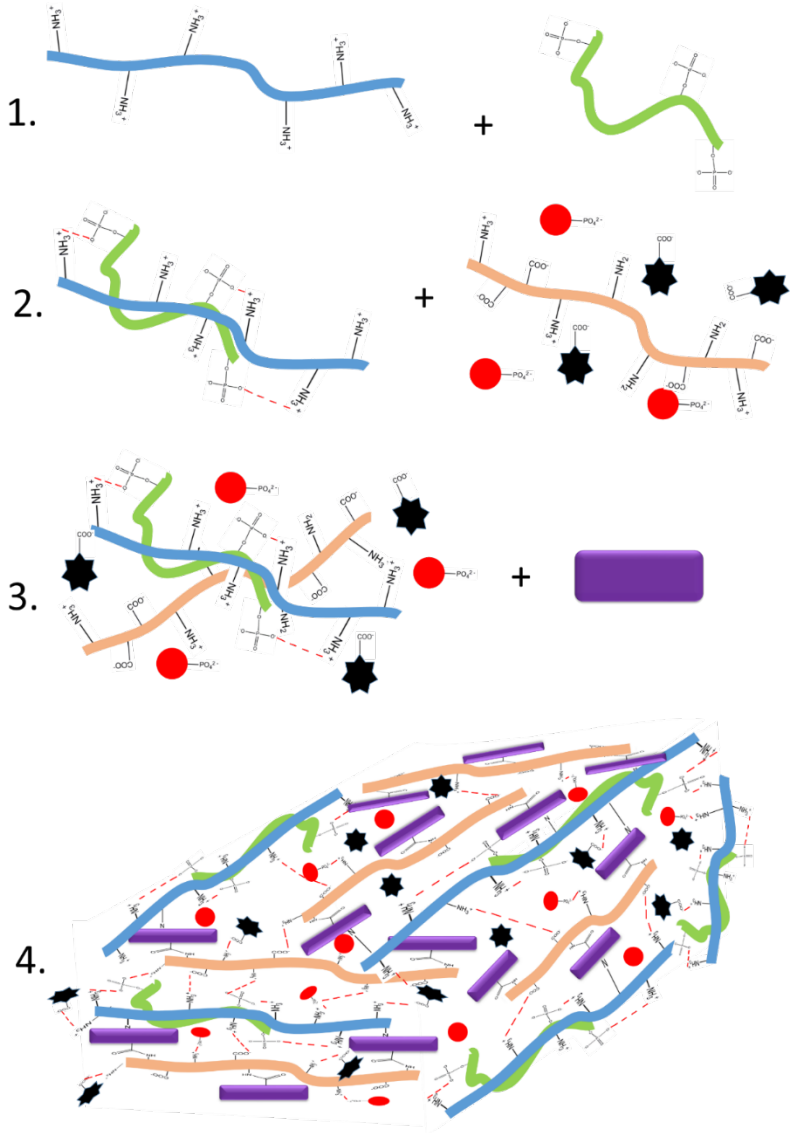

Figure 3.1. Schematic representation of interactions between polymers and crosslinkers i.e., Ionic interaction between CS NH groups and the phosphate groups from BGP (1), CS-BGP complex grafted onto GEL-MOX-DSP (2), Chemical crosslinking of CS-BGP-GEL-MOX-DSP by genipin (3), and A dual drug loaded thermoresponsive CS-GEL hydrogel (thermo-ocugel) (4)

3.3.2. Evaluation of molecular and chemical characteristics of biomaterials via FTIR

The FTIR analysis of the formulations and the starting materials are shown in Table 3.1 and Figure 3.2a. The starting materials exhibited their characteristic bands such as Amine (N—H), Amide I (C=O), and Amide II bands for gelatin and N—H bending and C=O stretching for chitosan. Crosslinking of chitosan with BGP resulted in the C=N wagging bond formation accompanied by major bands of amide stretching from CS and PO_4^{2-} bands from BGP. The presence PO_4^{2-} bands is indicative of the use of BGP in crosslinking chitosan. Furthermore,

the retained N—H bending and C=O stretching of CS bands and PO_4^{2-} bands in the spectrum of F1 is indicative of the ionic interaction between the two molecules. The lack of new bands in F1 is indicative of electrostatic stabilisation which does not result in new peak formation. This is similar to observations made by Kashi *et al*. [30]. Similarly, to F1 spectrum, the interaction of F2 lacked new bands except for those observed from the starting materials. The crosslinking of F2 with GEN resulted in a red shift (1636-1646 cm^{-1}) of the C=O band affected by the chemical interaction of GEN ester groups with that of CS amino groups. Furthermore, the interactions between the above molecules were also stabilised by hydrogen bonding thus facilitating the chemical shifts mentioned above. These findings correlate with previous discussions of chemical shifts resulting from hydrogen bonding between GEN and GEL and also the interactions between CS and GEN [24, 31, 32]. Furthermore, the O=P—OH band was present throughout the interactions which further attest to the involvement of BGP in the hydrogel fabrication. The drug molecules showed characteristic bands such as —C=O, C—F, P—O for DSP and C=O, C—N and Monofluorobenzene stretch for MOX. thermo-ocugel also resulted in further blue shift of C=O group due the interactions mentioned above. Furthermore, there were few bond transmittances that decreased such as the C=O, amide stretching and P=O bands while bands such as P—O and P—OH increased due to additional PO_4^{2-} groups from the drug molecules.

Table 3.1. Major FTIR bands of native materials and hydrogel formulations and comparative transmittance of formulations vs. native materials

Formulation	Wavenumber (cm^{-1})	Bond	Transmittance
GEL	3290, 3068	Amine (N—H)	
	2946, 2872	CH stretch	
	1627	Amide I bands (C=O)	
	1524	Amide II bands	
CS	3364, 3290	O—H	
	2872	CH	
	1655	C=O stretching	
	1581	N—H bending	
	1152	C—O—C bridge	
	1032	COO^- stretching	
BGP	3243	O—H	
	2872, 2946	CH_2, CH, O=P—OH	
	1673	O=P—OH	
	1469	P=O	
	1069	P—O	
	967	P—OH	

Table 3.2. Major FTIR bands of native materials and hydrogel formulations and comparative transmittance of formulations vs. native materials (ctd.)

Formulation	Wavenumber (cm^{-1})	Bond	Transmittance
F1	3188	O—H	Decreased
	2872, 9655	CH_2, CH, O=P—OH	Decreased
	1655	C=N wagging bond	Decreased
	1561	Amid stretching from CS	Increased
	1413	and C-N wagging bond	Increased
	1041	PO_4^{2-} band	
	958	P—OH	
F2	3262	O—H	Decreased
	2946, 2872	CH, O=P—OH	Decreased
	1636	C=O	Increased
	1552	Amid stretching from CS	
	1459, 1404, 1329	C-N wagging bond, and P=O	
	1255	P=O	Increased
	1041	P—O	
	958	P—OH	
DSP	3421	O—H	
	2937	CH	
	1720, 1673, 1618	—C=O	
	1302	C—F	
	1097	P—O	
MOX	1627, 1515, 1458	C=C stretch aromatic	
	874	C-H bending	
	1710	C=O	
	1329	C—N	
	1189	Monofluorobenzene stretch	
GEN	3401, 3216	O—H stretch	
	2946, 2835	CH bond	
	1683	C=O stretch	
	1618	C=C	
	1441	CH ester	
GEN	1311	C—O—C asymmetric stretch	
	1199	C—O stretch	
	1162, 1106	C—O—C stretching vibrations	
F3	3253	O—H stretch	Increased
	2946, 2881	CH, O=P—OH	Increased
	1646	C=O stretch	
	1552	Amid stretching from CS	
	1459, 1404, 1329	C-N wagging bond and P=O	
	1255	P=O	
	1041	P—O	
	958	P—OH	

Table 3.3. Major FTIR bands of native materials and hydrogel formulations and comparative transmittance of formulations vs. native materials (ctd.)

Formulation	Wavenumber (cm^{-1})	Bond	Transmittance
thermo-ocugel	3216	O—H stretch	
	2937, 2872	CH, O=P—OH	
	1636	C=O stretch from polymers and from drug molecules	Decreased
	1552	Amid stretching from CS, and C=O from MOX	Decreased
	1450, 1413	C-N wagging bond, —C=O from DSP and MOX	
	1255	P=O	Decreased
	1041	P—O	Increased
	958	P—OH	Increased

3.3.3. Evaluation of the diffraction pattern of biomaterials via XRD

To further confirm the structural integrity and interactions of native polymers, crosslinkers, drugs and hydrogels, XRD analysis was performed to investigate the crystallinity of the biomaterials (Figure 2b). The X-ray diffractogram of native CS exhibited a characteristic saccharide semi-crystalline phase at a 2θ of 21º with a shoulder-peak at 10 and an amorphous phase at 40. These results are similar to what was reported by Mndlovu *et al*. [28]. BGP showed crystalline phases at 6.12, 12.4, 13.98, 15.54, 16.46, 25.12, 25.54, 26.68, 27.54, 28.44, 29.56, 31.24, 32.36, 34.1, 36.44, 38.16º and a semi-crystalline phase at 20.52 degrees. The incorporation of BGP into the native CS resulted in the occurrence of an amorphous phase with a semi-crystalline phase at 23.1º and an amorphous phase at 44º representative of CS in F1. In addition to that there were crystalline phases at 6.14, 12.52, 28.48, 32.26 and 38.22º which were indicative of the presence of BGP in F1 (Figure 2b). The diffraction pattern of native GEL demonstrated an amorphous phase at 21.3º and 40º which was correlated to a typical α-helix and triple-helical structure as previously reported by Ki *et al*. and Badhe *et al*., respectively [33, 34]. F2 demonstrated a shoulder peak at 9.64, and amorphous phase at 22.54 and 43.1º which were indicative of the saccharide diffraction pattern. GEN displayed sharp phases at 11.78, 12.64, 16.8, 18.24, 19.12, 19.14, 19.84, 22.22, 23.9, 26.38, 27.92, 29.56, 30.58, 33.22, 37.4, 40.36, 44.04 and 46.9º which were indicative of the crystalline nature of genipin. F3 showed a shoulder peak at 9.26 and amorphous peaks at 22.78 and 43.18 respectively. The similarity of the diffraction pattern between F2 and F3 is indicative of the retained physical phase of F2 after crosslinking. The lower crosslinker (GEN) to polymer ratios used in F3 resulted in the polymer phases masking the diffraction pattern of the crosslinker. The thermo-ocugel displayed a semi-crystalline phase at 21.44º, an amorphous

phase at 48.3º, and crystalline phases at 6.3, 12.54, 13.64, 14.2, 15.8, 16.66, 26.76, 28.56, 32.28, 34.12, 37.26, and 38.44. The crystalline peaks displayed in thermo-ocugel were a contribution from the incorporated MOX with characteristic crystalline phases at 5.68, 8.34, 9.94, 11.48, 17.32, 26.44, 27.36, 29.1, and 40º and also DSP with characteristic crystalline phases at 4.54, 5.9, 7.76, 12.18, 13.88, 16.66, 20.12º.

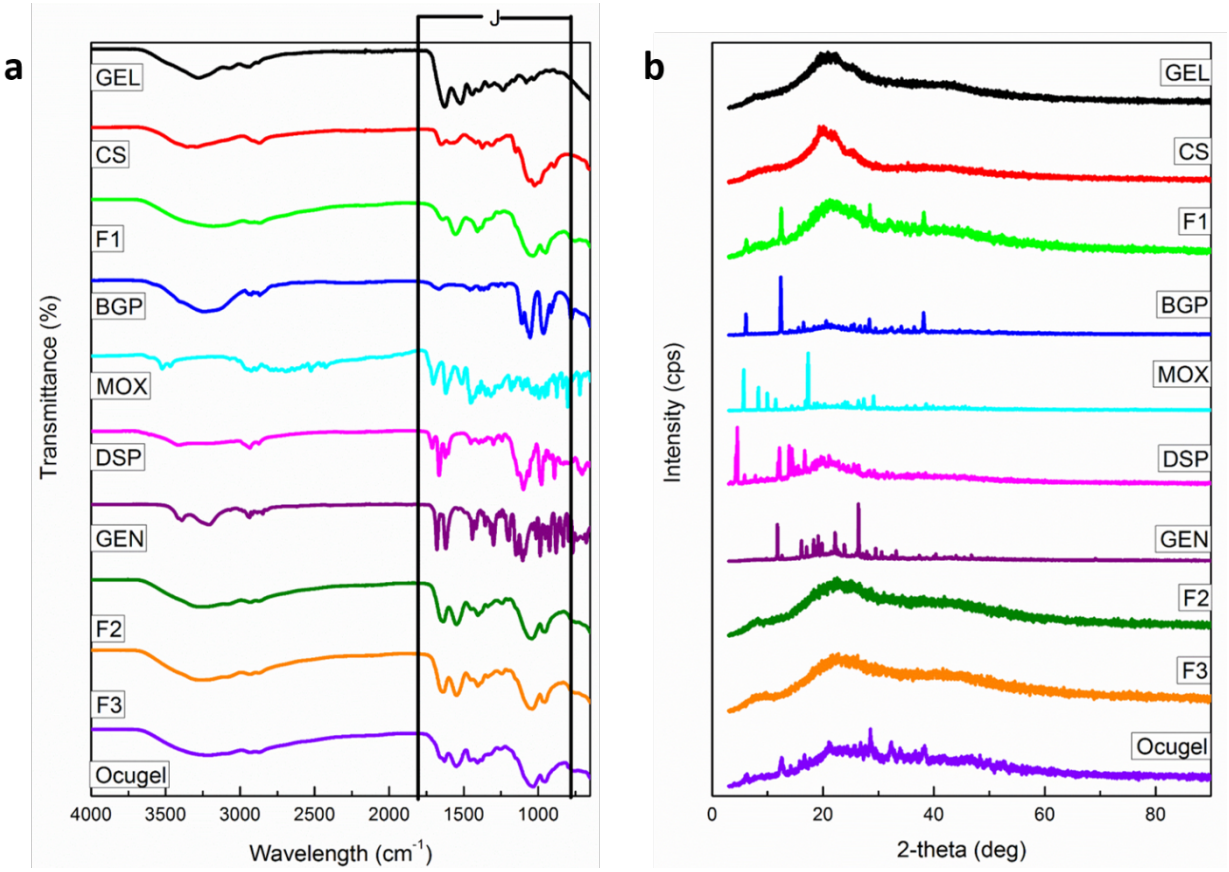

Figure 3.2. (a) FTIR analysis of biomaterials. “J” indicates the region where the interactions affect the chemical structure. (b) XRD analysis of biomaterials.

3.3.4. Thermostability assessment of native components and hydrogels

DSC thermograms of native polymers, crosslinkers, drugs and hydrogels are shown in Figure 3.3a. The DSC thermogram of CS demonstrated a broad endothermic peak at 92 °C corresponding to the dehydration of CS. A sharp exothermic peak at 305 °C corresponds to the degradation CS polysaccharide moiety. The thermogram of GEL showed a broad endothermic peak at 109 °C which can be attributed to the molecular dehydration, the molecule’s melting point was observed at 220 °C and degradation was observed from

~283 °C. The thermogram of BGP presented two distinctive endothermic peaks at 96 °C and 150 °C corresponding to molecular dehydration and melting point of BGP, respectively. The degradation for BGP was observed at 279 °C. The thermogram of GEN showed a sharp endothermic peak at 124 °C representative of the dehydration step. Furthermore, a slightly broad exothermic peak at 250 °C indicates degradation of GEN. These findings correspond to what was reported in literature [35-37].The F1 thermogram exhibited a broad endothermic peak at 83 °C indicative of the dehydration process, and the degradation started at 208 °C. However, there was a shift of the dehydration peak on F1 from 83 °C to 100 °C in F2 with degradation starting at 200 °C instead of the 208 °C seen in F1. The F1 crystal melted at 154 °C which would possibly be related to the presence of BGP. F2 had a higher melting point than the other hydrogels. Therefore, the incorporation of GEL increased the melting point to 195 °C. F3 showed no distinctive peaks. However, a broad endothermic phase was observed at 100 °C representing dehydration and the melting point for this hydrogel was observed at 179 °C. This insinuates that the addition of GEN does not alter the thermal properties of the hydrogels. The incorporated drugs exhibited endothermic phases at 113 and 91 °C for MOX and DSP, respectively. This is indicative of the drug molecule's dehydration. The melting points for these drugs were noted at 258 and 230 °C for MOX and DSP, respectively. The degradation for MOX was observed at 330 °C and the degradation for DSP was observed at 354°C. The thermo-ocugel presented with a broad endothermic peak at 91 °C indicating the point of dehydration. The melting point and degradation of the thermo-ocugel were observed at 150 and 338 °C, respectively. These thermostability properties were altered in comparison to the starting materials, which attest to the reactions that have occurred and changed the chemical structures of the biomaterials. The melting point and degradation temperatures are fairly high, indicating the stability of the hydrogels at elevated temperatures.

The thermal degradation behaviour of native polymers, crosslinkers, drugs and hydrogels was also compared by TGA with thermograms generated as shown in Figure 3.3b. Accordingly, CS showed saccharide-characteristic degradation of 42.48% within the temperature range of 281.57 and 361.78 °C. BGP depicted a bi-major degradation of 23.58% and 10.22% within the temperature range of 18.44 to 116.37 °C and 267 to 303.82 °C, respectively. F1 showed a poorly-defined degradation profile with slight weight loss of 12.02% within the temperature range of 63.96 to 96.61 °C which is representative of the presence of BGP and another weight loss of 20.69% within the temperature range of 255.73 to 347.7 °C which can be attributed to the presence of CS. GEL showed polymeric characteristic degradation with a major weight loss of 54.58% within the temperature range of 265.46 and 408.34 °C. F2 showed a major weight loss of 27.46% within the temperature range of 256 and 383.45 °C. The increased degradation ranges from 255.73-347.7 °C to 256-383.45 °C between F1 and F2 could be

attributed to the incorporation of GEL. At the temperature range of 179.58 to 256.12 °C, a 40.38% weight loss was observed for GEN. The incorporated drugs showed major weight loss over the temperature range 284.85 to 455.87 °C (51.54%) and 305 to 462 °C (49.12%) for MOX and DSP, respectively. F3 displayed a 32.94% weight loss over the temperature range of 242 to 374 °C whereas the dual drug-incorporated thermo-ocugel depicted a poorly-defined major degradation profile. However, a 17.33% slight weight loss was observed between 269 and 339 °C. The lack of a well-defined degradation profile in the thermo-ocugel may be due to the high proportion of CS-BGP in the thermo-ocugel which renders the overall degradation profile of the thermo-ocugel similar to the degradation profile of F1.

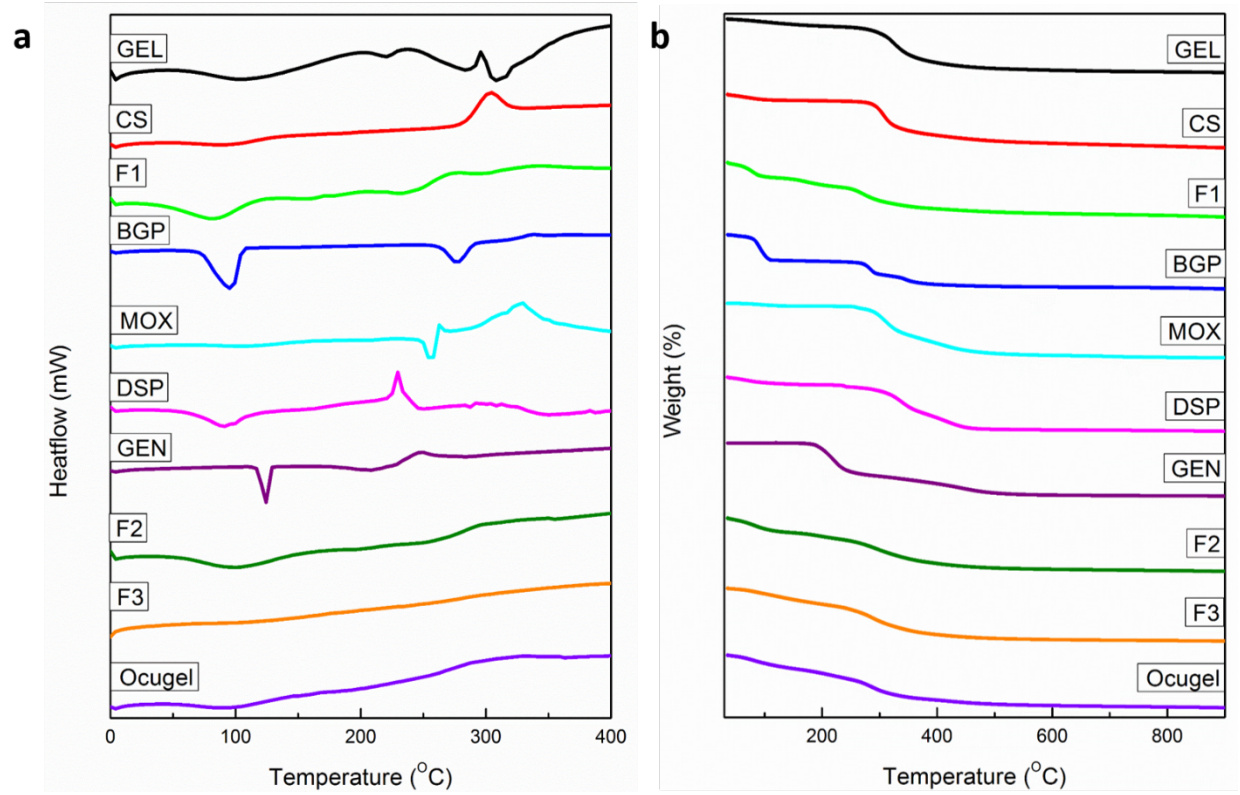

Figure 3.3. Thermo-stability properties of fabricated biomaterials. (a) DSC curves and (b) TGA thermograms of the biomaterials.

3.3.5. Assessment of mechanical properties of hydrogels

Thermoresponsive hydrogels have been widely researched for their application in tissue engineering for treatment of different injuries such as chronic ulcerated wounds and acute deep cut wounds, burns, and also for application in post-surgical interventions. Herein, a thermoresponsive hydrogel was designed for application in post-cataract surgery infection and inflammation. The rationale behind the design of *in situ*-forming thermoresponsive hydrogels is for the hydrogel to be able to respond to temperature changes and in this particular case, it should form a gel at physiological environmental conditions such as pH 7.0-7.4 and 37 °C,

thus forming a tightly interconnected, more rigid matrix structure for controlled release of the loaded drugs. The thermoresponsive nature of the hydrogel was assessed by following the storage modulus (G') over increasing temperature from 15 to 45 °C (Figure 3.4a). The storage modulus is linked to the gel strength/rigidity which indicates that the gel point is observed at the highest storage modulus.

The developed hydrogels displayed a gel point at the same temperature (32 °C). They also exhibited a similar gelling temperature range (25-38 °C), which indicates that gel formation is initiated at room temperature (25 °C), reaching the highest gelling point at 32 °C and remaining a gel up to 38 °C. Although the first three hydrogel formulations (F1, F2 and F3) showed a similar gelling temperature range (25-38 °C), the final hydrogel formulation (thermo-ocugel) showed a slightly narrower gelling range (25-35 °C). This slight difference in the gelling temperature range may be due to the addition of GEN and the drugs (DSP and MOX) which decreases the ratio of polymer to crosslinker available for interactions that would facilitate thermoresponsitivity of the hydrogel. The crosslinking of CS with BGP resulted in the formation of the thermoresponsive hydrogel with the highest rigidity (G') (F1). The addition of GEL to F1 decreased the rigidity of the hydrogel while maintaining the same gelling temperature. Crosslinking of F2 with GEN increased the storage modulus of the hydrogel. This attests to the ability of crosslinkers to induce/facilitate thermoresponsiveness in hydrogels. Incorporation of the drugs prior to crosslinking (GEN) on the other hand decreased the storage modulus of the hydrogel. This infers that the drugs interacted with the polymers thus limiting the number polymeric moieties available for interaction with the crosslinker (GEN) which in turn decreases the storage modulus of the formed gel.

The interactions between polymers, crosslinkers and drug molecules was also investigated by following the storage modulus of the hydrogels over time (Figure 3.4b). These studies investigated the changes in rigidity of the hydrogels associated with interactions between the employed biomaterials, crosslinkers and drugs. All hydrogels exhibited a high rigidity (final G'>1000 Pa). These characteristic features were followed independently of temperature and solely dependent on the interaction between the biomaterials, which altered the rigidity of the formulations. F1 interactions resulted in the lowest rigidity amongst the hydrogels. This was due to the fact that it remained a viscous gel over time. Similar observations were made for F2. Moreover, the interaction between CS and BGP is ionic and the same applies to the interaction between CS and GEL or F1 and GEL. The type of interaction could also have contributed to the hydrogel remaining a viscous gel.

Ionic interaction commences from the moment the molecules are mixed thus the rigidity increased from the starting point whereas chemical crosslinking is only evident after a specific period from the starting point thus the changes in rigidity were only observed after 5 hours. Introduction of GEN as a crosslinking agent increased the rigidity of the gels drastically to G'>15 kPa compared to non-crosslinked gels which had G'<2 kPa (Figure 3.4b). This infers that chemical crosslinking has a high potential of producing a stiffer hydrogel than ionic crosslinking. F3 exhibited the second highest rigidity and was only surpassed by the thermo-ocugel hydrogel. The sharp increase in storage modulus on the highly rigid hydrogels was observed from 5h30 which correlates with the reported genipin crosslinking time [38]. The increased rigidity observed in the GEN crosslinked hydrogels correlates with the 3D architecture of the thermo-ocugel as depicted in Figure 3.1d above.

The final hydrogel formulation (thermo-ocugel) mechanical properties are shown in Figure 3.4c. The storage modulus (G'), loss modulus (G") and loss tangent (tan d) represents the hydrogel's elasticity/rigidity, viscosity and sol/gel state. The storage modulus and the loss modulus proportionally increased over time which inferred the presence of visco-elastic properties. The slight increase in loss tangent indicates that the hydrogel transitioned from the sol state to the gel state. These properties are crucial in the sense that they emulate the physiological tissues in terms of presenting visco-elastic properties and highlights the ability of the thermo-ocugel to exist in a gel state to prevent dilution by the tears. The increase in viscosity and rigidity of the hydrogel was inversely proportional to the sample height. This implies that as gel formation occurs within the hydrogel it becomes physically rearranged to form a stable and stiffer contracted gel. This is also supported by the increasing shear complex modulus (G*) over time.

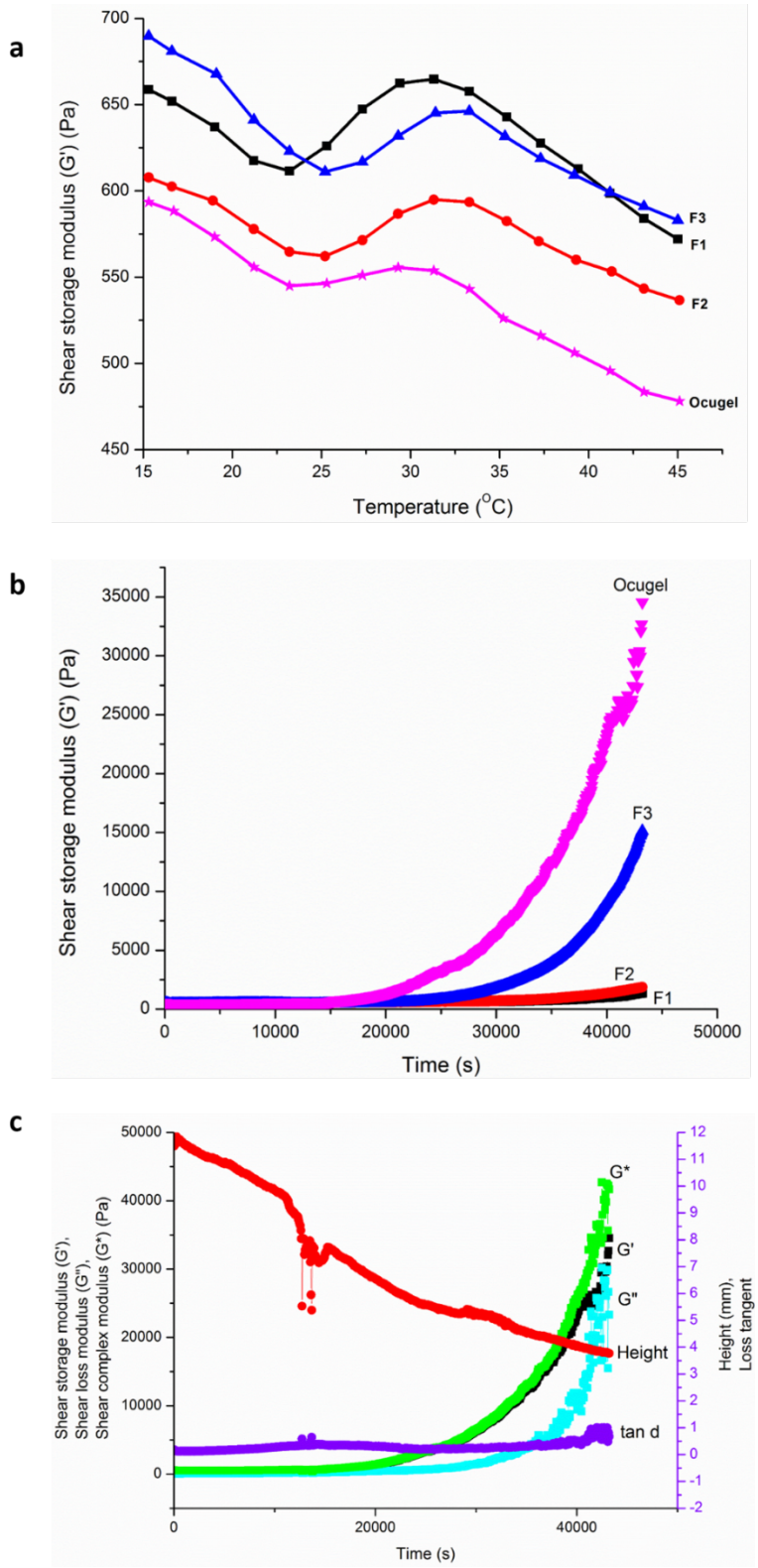

Figure 3.4. Rheological properties of the hydrogels. (a) thermo-responsive properties of the hydrogels and (b) shear storage modulus as a function of time for all hydrogels, and (c) time-lapse of the thermo-ocugel which assesses the interaction between the polymers and the crosslinkers.

Rheological properties of the hydrogels were assessed to confirm the mechanical properties of the hydrogels highlighted in the previous section. The apparent yield stress of the hydrogels provided more information about the shear compression that can be applied to them before deformation commences. The hydrogels yield stress increased with their rigidity. The shear yield stresses of hydrogels were 16.73, 7.812, 64.24, and 117.2 Pa for F1, F2, F3, and thermo-ocugel, respectively. The rigidity has already been discussed in detail in the previous section and it increased drastically after the introduction of GEN as a crosslinker. The direct proportionality of the yield stress with the hydrogel's rigidity was also previously reported [28, 39].

The visco-elastic properties of the hydrogels were also evaluated by following the elastic modulus G' as a function of shear stress (Figure 3.5). All hydrogels exhibited visco-elastic properties which were highlighted by the presence of resistance to deformation as the shear stress increased (Figure 3.5a). However, F1 and F2 displayed a decrease in elastic modulus as the shear stress increased which inferred that they were more susceptible to deformation compared to their chemically crosslinked counterparts F3 and thermo-ocugel which showed an increase in elastic modulus over increasing shear stress. The high resistance to deformation on F3 and thermo-ocugel may be attributed to high rigidity induced by chemical crosslinking of hydrogels with GEN. Chemical crosslinking changed the 3-D architecture of the hydrogels and formed more stable and rigid structures which in turn induced resistance to deformation in F3 and the thermo-ocugel.

The shear viscosity evaluation of F3 and the thermo-ocugel hydrogels exhibited a highly susceptible unthickening of the gels over shear rate compared to their ionically crosslinked counter parts (F1 and F2) (Figure 3.5b). This unthickening behaviour of F3 and the thermo-ocugel hydrogels further suggest that the hydrogels were more structured and rigid. Thus, when the shear rate increases, the viscosity of the gels drastically. However, in the case of less structured complexes (F1 and F2), the shear viscosity is not heavily affected by the increasing shear rate. Thus, the hydrogels (F1 and F2) only showed a gradual decrease in shear viscosity as the shear rate increases.

The hydrogels' thermoresponsitivity was also investigated by following the shear elastic modulus and shear viscosity with increasing temperatures (Figure 3.5c). The observations made on the shear elastic modulus and shear viscosity correlated with those reported in the previous section. F1 exhibited a gradual increase in both shear elastic modulus and shear viscosity from 25 °C, and also showed a sharp increase in both parameters from 35 °C up to 44 °C. Similar observations were also made in the other hydrogels F2, F3 and thermo-ocugel.

However, F1 showed a major increase in both shear elastic modulus and shear viscosity compared to the other hydrogels. This performance may be due to the point that F1 was the only thermoresponsive biomaterial in the hydrogel. Thus, the shear elastic modulus and shear viscosity displayed sharp increase in F1 compared to the other hydrogels. The limited increase in both shear elastic modulus and shear viscosity in F2, F3, and the thermo-ocugel hydrogels was due to the high rigidity as a result of crosslinking. The shear elastic modulus and shear viscosity behaved similar in each hydrogel. However, both parameters were higher in the thermo-ocugel which further attest to the highly rigid 3-D architecture of the gel.

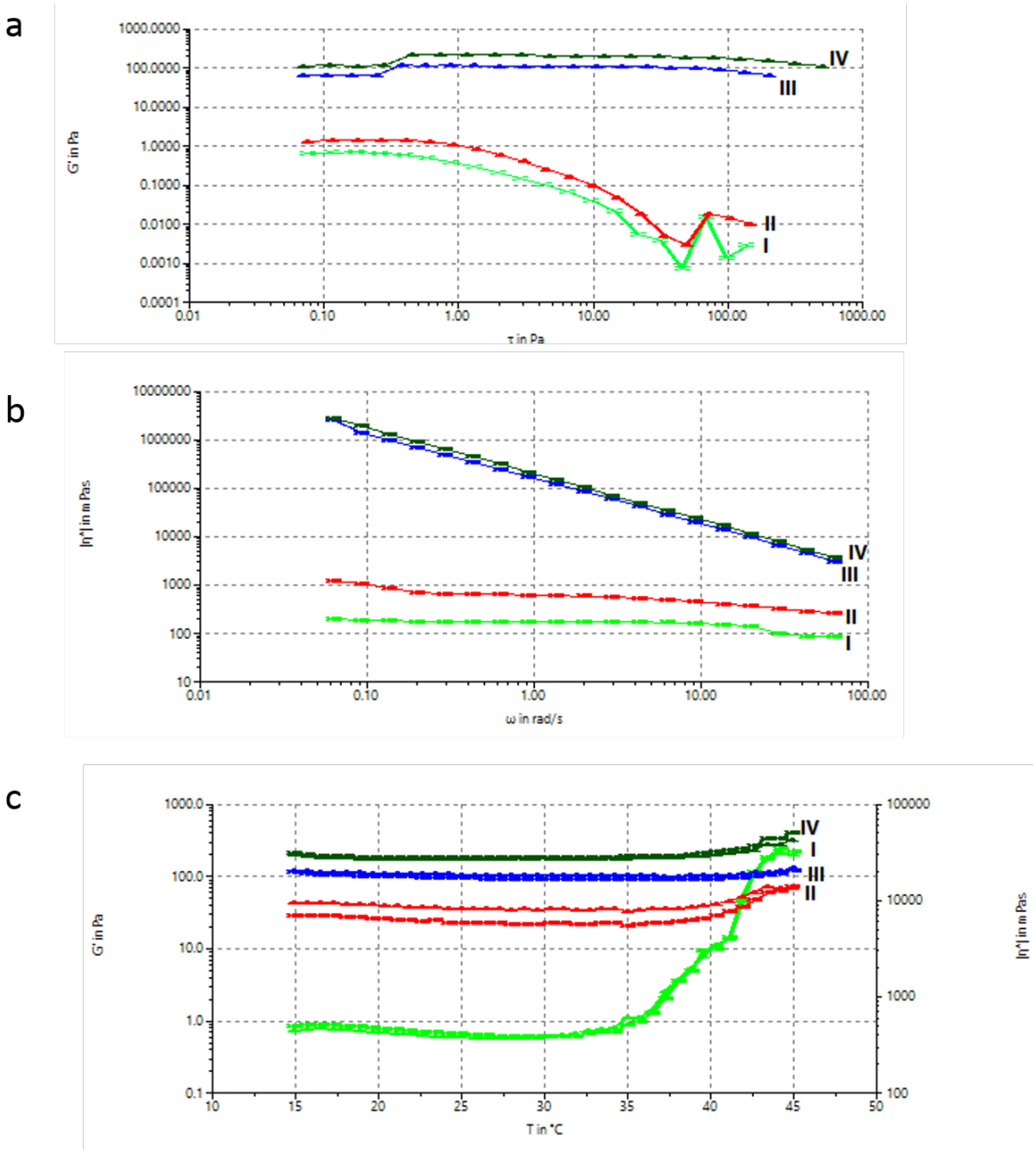

Figure 3.5. Mechanical properties of the hydrogels (I) F1, (II) F2, (III) F3, and (IV) thermo-ocugel. (a) Represents the shear elastic modulus as a function of shear stress, (b) Shear

viscosity and (c) shear elastic modulus and shear viscosity as a function of temperature of the hydrogels.

3.3.6. Analysis of drug loading and release from the hydrogel

The UV-Vis absorptive properties of the thermo-ocugel were analysed over 200 – 500 nm to obtain the absorption spectra. The incorporated drugs in the thermo-ocugel showed two peak maxima at 242 and 288 nm for DSP and MOX, respectively. In this study a 0.1% $^{w}/_{v}$ and 0.5% $^{w}/_{v}$ were used for DSP and MOX, respectively. These concentrations were used due to their reported therapeutic effects [40, 41]. The dual drug loading studies were done in STF. The drug loading was 1.87% and 6.14% for DSP and MOX, respectively. The drug release studies were performed in STF at pH 7.4, thus assessing the thermo-ocugel's ability to present a controlled simultaneous release of DSP and MOX in a simulated ocular environment.

The thermo-ocugel showed a burst release of 52.75 ± 3.12% and 60.94 ± 8.31% in 12 hours, which increased to 76.61 ± 5.91% and 81.71± 4.85% in 3 days followed by a steady release of 0.53 ± 0.15% and 0.81 ± 0.26% per week for DSP and MOX, respectively (Figure 3.6). Previous investigations have indicated a complete release of 81% in 12 hours for MOX [42] and 80% in 48 hours for DSP from intraocular systems [43]. The prolonged drug release observed in the current study attests to the capabilities of the thermo-ocugel to retain the drugs for a prolonged period. Given that the drug release was evaluated from the point where all the thermo-ocugel components were mixed in viscous liquid state, the burst release was notable. This burst release indicates an incomplete crosslinking by GEN where the thermo-ocugel was still in the transition phase from a viscous liquid to a gel state. The burst release was followed by a steady release which was indicative of release from the crosslinked thermo-ocugel. These findings correlate with the mechanical properties of the thermo-ocugel where the thermo-ocugel became more rigid from five hours of GEN crosslinking as visualised in Figure 4b. The results also correlate with the high fluid uptake of the thermo-ocugel in the first 3 hours of fluid exposure as depicted in Figure 3.8. The therapeutic effect of MOX is reported to occur from a minimum of 0.2 µg/mL or >MIC_{90} for aerobic Gram-positive and Gram-negative pathogens responsible for causing eye infections [44]. In this study, the sustained release phase was observed to be attained at 3 days at 81.0 ± 26.0% release (corresponding to 0.03 mg/mL), which is sufficient to induce a therapeutic effect. DSP's anti-inflammatory effects are reported from concentrations as low as 0.5164 µg/mL (1 µM); herein, achieved at 3 hours at 53.0 ± 15.0% release (corresponding to 6.61 µg/mL) of DSP was observed during the sustained release phase thus indicating that it would maintain an adequate therapeutic effect [42], further confirmed via subsequent *in vivo* investigations. DSP was also shown to be cytocompatible

even up to a concentration of 1 mg/mL [43]. The high burst release (>50%) and the sustained release of <1% per week is indicative that the incorporated drugs would have enhanced therapeutic effects at the point of potential infection and early stages of wound healing and also present sufficient concentrations to maintain the effective therapeutic levels which is of essence in a successful treatment of post-cataract surgery infection and inflammation.

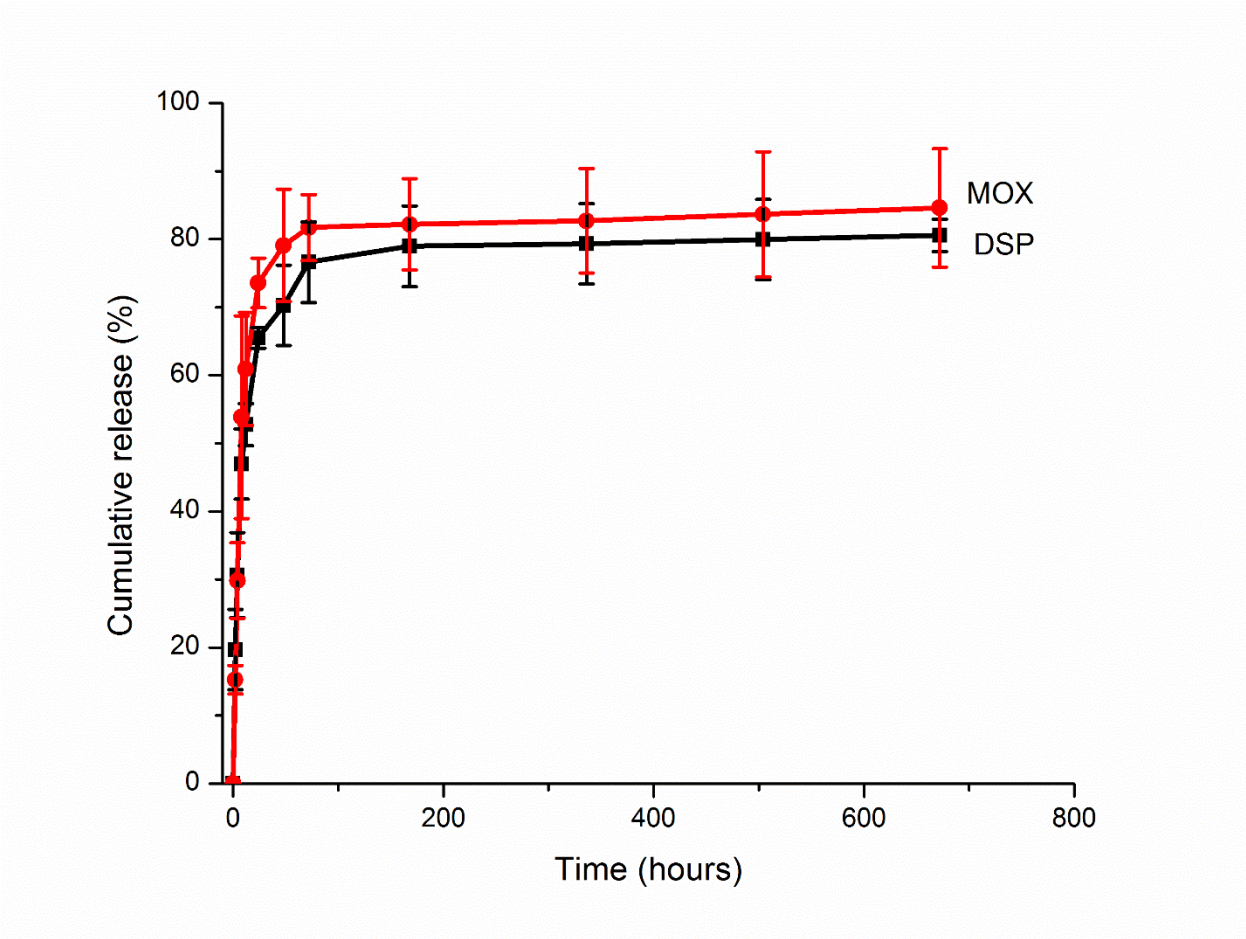

Figure 3.6. Cumulative release of DSP and MOX from the hydrogel under physiological conditions (37 °C, pH 7.4).

3.3.7. Morphological features of the lyophilized hydrogels

The SEM images of the lyophilized hydrogels are depicted by Figure 3.7. All images indicate the top-view surface morphology of the hydrogels. All formulations were porous with different shapes and sizes. F1 displayed a flatly aligned elongated sheets with slight consistent particle deposition on the surface (Figure 3.7a). F2 morphological appearance was similar to that of a dried riverbanks with less pronounced particle deposition on the surface (Figure 3.7b). The change in the morphological appearance from F1 to F2 can attributed to blending CSBGP with GEL. F3 had a single sheet with irregular particle deposition and the surface morphology

assumed that of a mountain with caves (Figure 3.7c). This morphological appearance had less pores which could be the result of GEN crosslinking. The morphological appearance of the thermo-ocugel depicted a single sheet with thread-like structures on the surface and less pronounced particle deposition (Figure 3.7d). The decreased porosity in thermo-ocugel morphology compared to the other hydrogels could have been contributed by the dual crosslinking approach and drug incorporation.

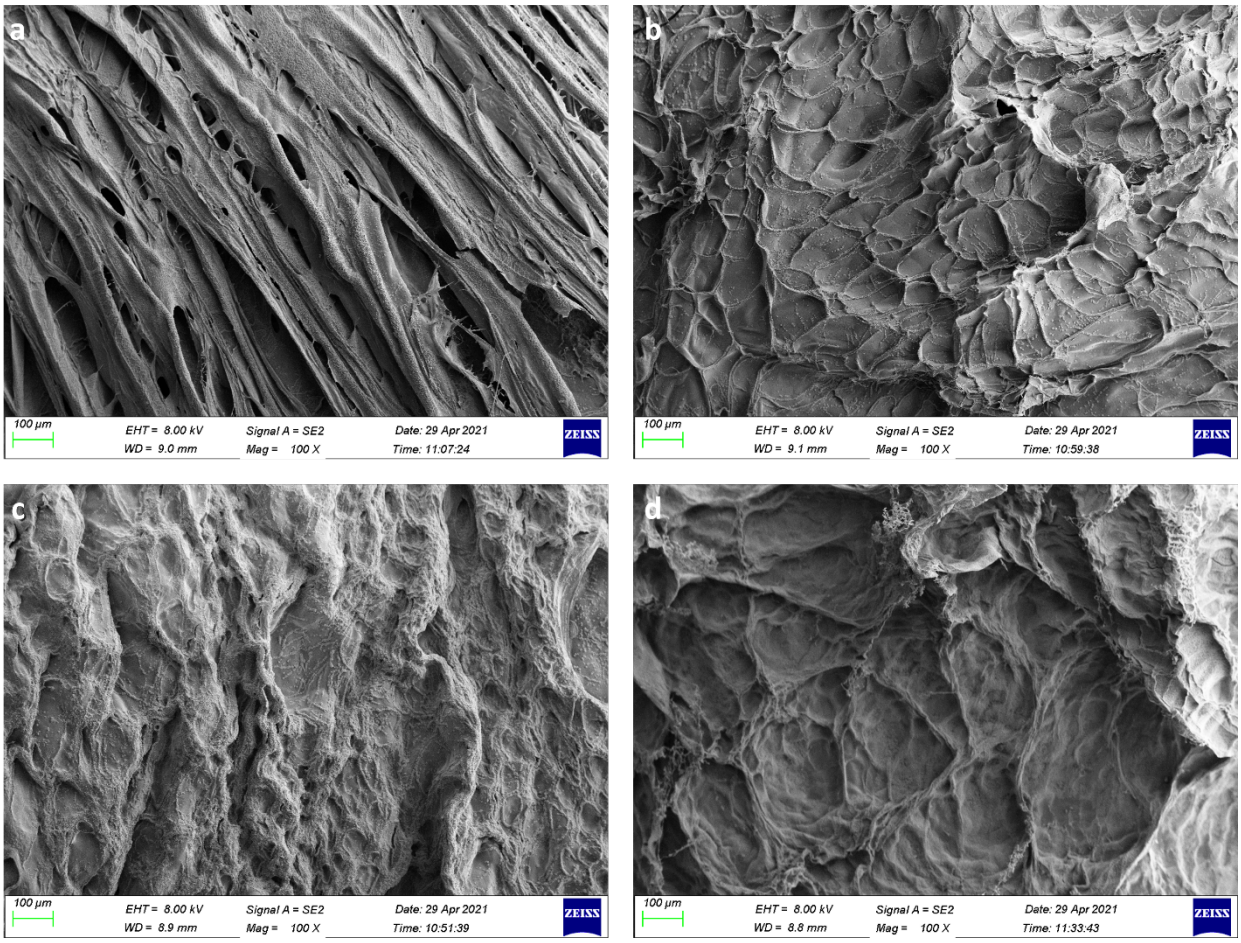

Figure 3.7. SEM images of the fabricated hydrogels. (a) F1, (b) F2, (c) F3, and (d) thermo-ocugel.

3.3.8. Fluid uptake and erosion of the thermo-ocugel

Hydrogels have been widely researched in terms of drug delivery because they are able to absorb a number of different substances within their cross-linked matrix [45]. This speaks to their ability to swell when immersed in water or an aqueous environment. Figure 3.8 depicts how the employment hydrophilic polymers, CS and GEL aids in hydrogel's capability to absorb water over time. The thermo-ocugel which is composed of CS, BGP, GEL, MOX and DSP

displayed a rapid fluid uptake of 126.02% in the first 3 days followed by a gradual increase up to 198.72% in 28 days. This could be attributed to the use of GEN and BGP as a crosslinking agents. This correlates with reports of increased fluid uptake in crosslinked chitosan compare to non-crosslinked chitosan [28]. The rapid fluid uptake within the first 3 days highlights the ability of the hydrogel to hydrate from the surface and the fluid slowly moves towards the bulk of the hydrogel due to slight fluid saturation.

The erosion of the thermo-ocugel was studied by monitoring the weight loss of the hydrogel under physiological conditions and slightly agitated the samples via the use of the orbital shaker at 37 °C at a speed of 50rpm for 28 days. Figure 7 illustrates the thermo-ocugel weight loss as a function of time. The thermo-ocugel exhibited a slow degradation rate on the first 3 days (1.5 ± 0.10% erosion) whereas a slightly high degradation rate was observed between 3 and 14 days (16.58 ± 0.32% erosion). As noted in the fluid uptake behaviour of the thermo-ocugel, a large amount of fluid was absorbed in the first 3 days. This allowed the hydrogel to start degrading after a large proportion of the hydrogel was absorbed. The high degradation rate after 3 days is indicative of bulk degradation mechanism. The bulk degradation was followed by a steady degradation which implied that bulk of the hydrogel had already degraded. The thermo-ocugel displayed a slow degradation with 18.20% ±0.06 degradation in 28 days. This slow degradation profile of the thermo-ocugel may be attributed to the use of GEN and BGP as crosslinkers. Previous studies have reported that an increase of GEN concentration resulted in a slower degradation and mechanical strength [46]. Similar results were also reported by Song *et al*. [24], who investigated the effect of GEN concentration on the GEL weight loss as a function of time. A rapid weight loss was observed for the CS-GEL/BGP hydrogels without GEN (0 µg/mL) whereas, a 43% weight loss was observed for a lower GEN concentration (10 µg/mL) with a 25% weight loss for a higher GEN concentration (100 µg/mL). The continually controlled, but fairly constant rate of degradation of the thermo-ocugel is proposed to aid in the elimination of the requirement for constant injections which could deter patient compliance. This addresses the problem encountered in most ocular drug delivery systems whereby patients complain about the frequency of dose administration and expenses incurred.

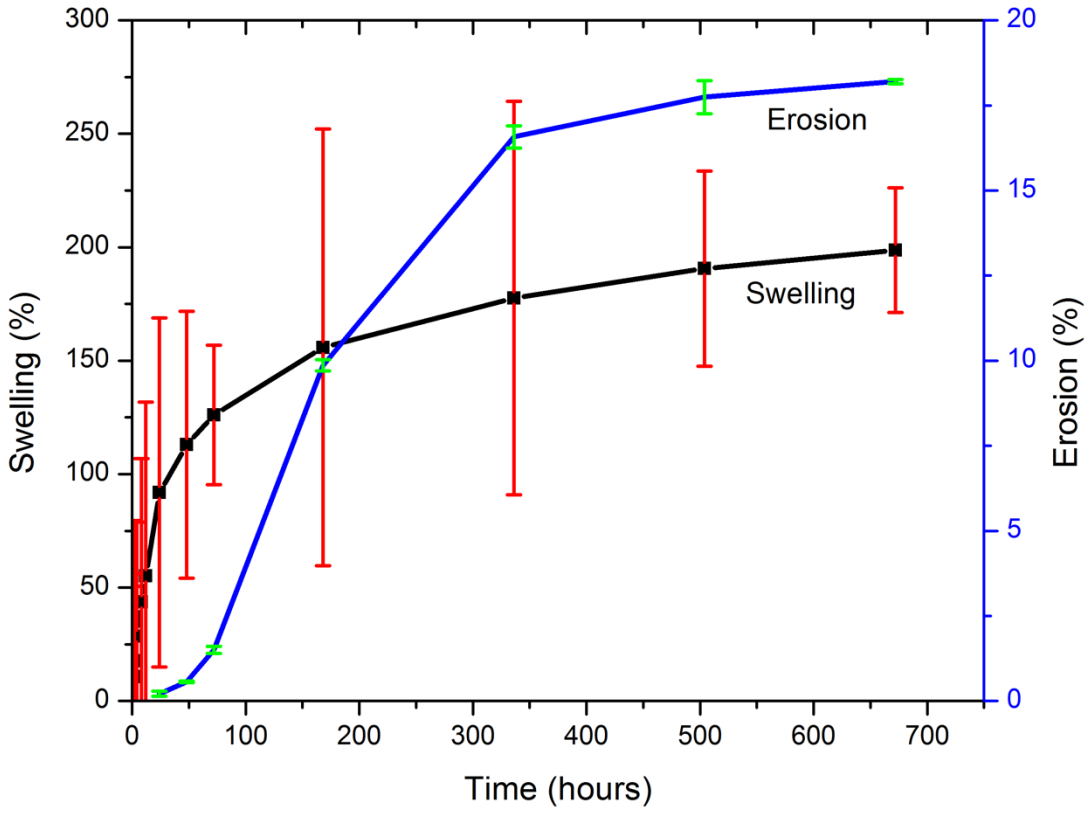

Figure 3.8. Erosion and swelling of hydrogel under simulated physiological conditions (pH 7.4 STF, 37 °C, agitation speed of 50rpm).

3.3.9. Relationship between cumulative release with fluid uptake and erosion of the thermo-ocugel

The kinetic models from the thermo-ocugel are represented in Table 3.2. The release kinetics for both MOX and DSP were best depicted by the Korsmeyer-Peppas model (R^2 = 0.6658 and 0.9092 for MOX and DSP, respectively) and Higuchi model (R^2 = 0.4824 and 0.5840 for MOX and DSP, respectively). The Korsmeyer-Peppas model showed the best fit for both MOX and DSP from the thermo-ocugel drug release data (<60%) following a plot of log cumulative % drug release as a function of log time. This model highlighted a non-Fickian (Case II) model release mechanism. The zero-order and first-order kinetics are slightly applicable for both MOX and DSP with correlation coefficient (R^2) ranging from 0.3091 to 0.5070 for both MOX and DSP, in accordance with the linear models. The first-order relationship infers that most of the drug is released in the first 3 days with slight degradation of the thermo-ocugel; and zero-order is coupled with a lower drug release rate and increased surface erosion of the thermo-ocugel. This indicates that the thermo-ocugel presents desirable release kinetics for treatment

of the initial potential infection and inflammation that can occur post-surgery as a result of adequate drug concentrations, which are rapidly attained following the burst release as depicted by Figure 3.6. The drug release was further correlated with fluid uptake (Figure 3.9). There was a direct proportionality between drug release and fluid uptake. The drug release increased with enhanced fluid uptake by the thermo-ocugel. This indicates that as more of the fluid permeates into the core of the hydrogel, the more drug would be allowed to leave the hydrogel. This high drug release and high fluid uptake phenomena was observed on the within the first 12 hours of fluid exposure. However, as the thermo-ocugel get saturated with fluid, a slow fluid uptake was observed which resulted in a slow drug release (Figure 3.9a). The swelling-cumulative release behaviour exhibited a biphasic relationship with the initial phase mimicking a first-order relationship observed in the first 3 days followed by a zero-order relation from 3 days to 28 days (Figure 3.9b).

Table 3.2. *In vitro* kinetics analysis of drug release from the thermo-ocugel

Form	Zero Order Model (R^2)	First Order Model (R^2)	Higuchi Model (R^2)	Korsmeyer-Peppas Model (R^2)	Hixson-Crowell Cube Root Law (R^2)
MOX	0.3091	0.4345	0.4824	0.6658	0.3903
DSP	0.3916	0.5070	0.5840	0.9092	0.4681

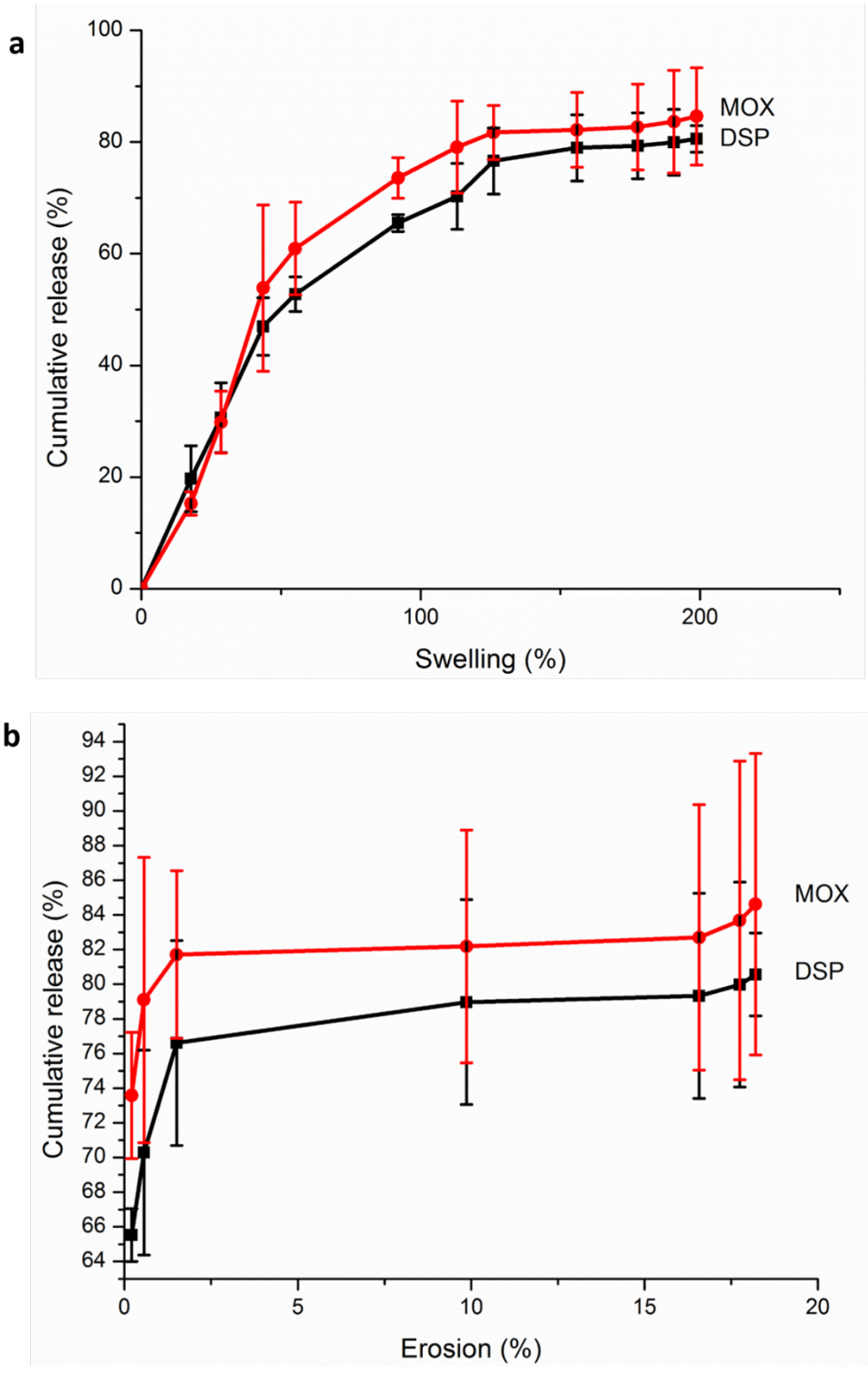

Figure 3.9. (a) Cumulative release as a function of swelling and (b) cumulative release as a function of erosion of hydrogel under physiological conditions.

3.4. Concluding Remarks

This study assessed the potential of dual drug-loaded thermoresponsive chitosan-gelatin hydrogel to form a novel thermo-ocugel for the potential treatment of post-cataract surgery infection and inflammation. The employed crosslinkers were crucial in modifying and enhancing polymer properties such as thermoresponsitivity and sustained drug release. Furthermore, the thermo-physical properties were improved along with additional thermo-ocugel properties such as increased fluid uptake, drug retention, improved rheological properties and controlled biodegradation rate, which positively affected performance of the thermo-ocugel. Therefore, the desired properties render it a promising candidate for the treatment of ocular infections and inflammation resulting from surgical procedures.

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CHAPTER 4

In vivo investigation of the dual drug-loaded thermoresponsive thermo-ocugel for potential treatment of post-cataract surgery infection and inflammation

4.1. Introduction

A cataract is an ocular condition which is characterized by the loss of lens transparency due to opacification of the lens as a result of oxidative stress [1]. Cataracts are characterized as age-related cataracts, paediatric cataracts, and cataracts secondary to other medical conditions, depending on the aetiology. The most frequent type of cataract in adults is an age-related cataract, which appears between the ages of 45 and 50 [2]. If left ignored, cataracts can cause vision to worsen thereby seriously affect patient's quality of life. In some patients, vision can be corrected by spectacles or contact lenses. However, in majority of patients presenting with cataracts, the primary treatment is surgical removal of the lens. In many countries, cataract surgery is still one of the most cost-effective therapies and the most regularly performed technique. Despite advances in surgical technology and techniques over the last decade, successful surgical outcomes still require a complete preoperative evaluation, precise intraocular lens power estimation, and appropriate intraoperative and postoperative treatment [1].

Antibiotics and corticosteroids, in combination, are recognized to be excellent therapeutic remedies for post-cataract surgery therapy [1]. Moxifloxacin hydrochloride is one of the most widely used antibiotic with bactericidal action involving the inhibition of DNA gyrase processes and causing bacterial destruction. Moxifloxacin therapeutic effectiveness has made it popular in the treatment of ocular conjunctivitis, postoperative endophthalmitis, and bacterial keratitis [3]. Moxifloxacin also has a broad spectrum of activity, higher potency, delayed antibiotic resistance, and improved tissue penetration [4]. As a result of this drug's short half-life in the aqueous humour (about 2.2 hours), repeated dosage instillation (3-4 drops 3 times daily) is required to obtain effective concentrations. [5]. Dexamethasone, a synthetic glucocorticoid derivative, is a powerful and long-acting glucocorticoid [6]. Dexamethasone sodium phosphate was shown to have the highest potency and efficacy of the corticosteroids used in ocular therapy [7]. The mechanism of action of dexamethasone follows the inhibition of prostaglandins synthesis and other inflammation mediators [8]. Dexamethasone is mainly used in ocular therapeutics for treatment of inflammations caused by infections, injury, surgery, or other conditions in the eyes [6]. However, the use of dexamethasone is also limited by its short half-life of 3-6 hours in the aqueous humour, which similarly to moxifloxacin, would

make frequent administration mandatory for attainment of effective concentrations in ocular tissues [9]. Thus, the use of hydrogels composed of biodegradable polymers has been reported to have excellent entrapment capabilities that aid in the circumvention of multiple drug drainages and ocular barriers [10].

Mucoadhesive polymers have gained popularity for use in ocular drug delivery due to their reported ability to increase drug retention time and ocular surface mucoadhesion [11]. Chitosan, a linear polysaccharide, is a biodegradable hydrophilic mucoadhesive polymer that is expected to retain and stabilize tear fluids on the surface of the eye, limiting drainage and extending precorneal retention [12]. Since it is a natural polymer, chitosan has been shown to be a non-toxic, biodegradable, and biocompatible biomaterial. It also has a wide range of biomedical applications, including drug delivery, drug targeting, wound healing, tissue engineering, and other nanobiotechnology-related disciplines [11, 13]. Hence, chitosan was chosen as a biomaterial to facilitate delivery of moxifloxacin and dexamethasone to ocular tissues. Composites of chitosan with proteins has been reported to offer good biocompatibility and excellent biomimetic strategies [14]. Moreover, drug delivery systems made of biodegradable biomaterials such as protein-derived polymers (gelatin) and polysaccharides (chitosan) can act as efficient drug delivery vehicles for controlled and targeted release [15]. Gelatin is obtained by hydrolysis of animal collagen, which makes it an excellent candidate for this study due to advantages such as high biocompatibility, less antigenicity, and accessible functional groups that allow for chemical modifications such as crosslinking [14].

The previous chapter focused on the design and fabrication of moxifloxacin HCl and dexamethasone sodium phosphate in an *in situ* forming chitosan-gelatin hydrogel (thermo-ocugel) with optimal mechanical properties, dual drug incorporation, and fluid uptake. The observations presented in previous chapters were built upon to investigate possible translational performances such as *in vivo* compatibility and ocular tissue response to the introduction of the developed thermo-ocugel in the New Zealand white rabbit ocular model through the histomorphological assessment of cellular infiltrates to corroborate the feasibility and clinical viability of the intracamerally injected thermo-ocugel in human patients. Concentrations of both MOX and DSP in the normal rabbit eye following intracameral injection was also undertaken for assessment of the preclinical efficacy of the thermo-ocugel.

The use of intracameral injections represents some of the strategies explored to overcome the challenges associated with drug delivery to the ocular anterior segment such as limited bioavailability and frequent instillations. The intracameral route offers targeted drug administration thereby allowing adequate amount of drug levels to reach therapeutic

concentrations at the site of action [16]. Furthermore, this route bypasses rate limiting factors such as corneal barriers, conjunctival barriers, and blood-aqueous-barrier. It therefore necessitates a lower dosage [6]. Intracameral injections have been reported to easily deliver drugs to the anterior segment with high efficiency. However, with all the advantages of intracameral delivery, toxic anterior segment syndrome (TASS) and corneal endothelial cell loss still remain a challenge [6]. Another drawback associated with the use of intracameral injections is the possible need for reconstitution of the therapeutic drug, which may predispose the patient to toxicity if done incorrectly [17]. Despite all these drawbacks, intracameral injections are still preferred for ocular post-operative care due to reasons such as targeted delivery to the site of action, bypassing of ocular barriers, increased bioavailability, elimination of frequent instillations, and prevention of possible infection risks.

4.2. Materials and Methods

4.2.1. Materials

For the thermo-ocugel (Low molecular weight chitosan, Gelatin (PH EUR), β-Glycerophosphate disodium salt hydrate, genipin, moxifloxacin HCL and dexamethasone sodium phosphate were purchased from Sigma-Aldrich (St. Louis, MO, USA)). The Central Animal Services at the University of the Witwatersrand, Johannesburg, South Africa, provided anaesthetics, povidone iodide, topical antibiotics (chloramphenicol ointment), and euthanasia drugs (pentobarbitone) for administration to the rabbits. The rest of the reagents were of the highest purity and analytical grade. Table 4.1 outlines all of the drugs and their dosages for administration to the rabbits.

Table 4.1. Drugs and recommended dosages as administered to rabbits

Drug/ formulation	Therapeutic classes	Route	Dose	Frequency
Thermo-ocugel (0.5% $^{w}/_{v}$ MOX and 0.1 $^{w}/_{v}$ DSP)	• antibiotic • anti-inflammatory	Intracameral	0.1 mL	Once-off injection
Tetracaine HCL 0.5% $^{w}/_{w}$	• topical anaesthetic	Topical	1-2 drops	Prior to intracameral thermo-ocugel injection
Xylazine HCL	• sedative, anaesthetic • muscle relaxant • analgesic	I.M.	10 mg/kg	Prior to intracameral thermo-ocugel injection

Table 4.2. Drugs and recommended dosages as administered to rabbits (ctd.)

Drug/ formulation	Therapeutic classes	Route	Dose	Frequency
Ketamine HCL	• dissociative anaesthetic	I.M.	40 mg/kg	Prior to intracameral thermo-ocugel injection
Chloramphenicol ointment 1% $^{w}/_{w}$	• antibiotic	Topical	Thin layer to lower lid	Post intracameral thermo-ocugel injection
Sodium pentobarbitone	• euthanasia	I.V.	>50 mg/kg	Once-off on euthanasia

4.2.2. Analysis of the *in vivo* behaviour of the thermo-ocugel in New Zealand Albino Rabbits

The Animal Ethics Committee of the University of the Witwatersrand granted ethics permission for the subsequent *in vivo* investigation, Ethics Clearance No. 2020/03/05/C.

4.2.2.1. Sterilisation of the thermo-ocugel for surgical preparation

The thermo-ocugel was sterilised under the UV lamp for 30 minutes to kill any potentially toxic microorganisms that may have been present.

4.2.2.2. *In vivo* study design

Male and female New Zealand white rabbits weighing 1.5-2.5 kg (free of any symptoms of ocular irritation or gross abnormalities) were used to conduct *in vivo* tests in the following investigations. The rabbits were randomly assigned to the experimental (thermo-ocugel), placebo (drug-free) and control groups. The minimum animal sample size for testing was ascertained from the specifications of the Animal ethics Committee of the University of the Witwatersrand for statistical significance where n=3 for placebo and n=3 for experimental groups, respectively.

All procedures followed the guidelines set out by the Association for Research in Vision and Ophthalmology (ARVO) for animal use in ophthalmic and vision research. The rabbits were kept at room temperature with a 12-hour light/dark cycle (light from 6 a.m. to 6 p.m.) and were fed a regular rabbit diet with free access to water. The rabbits were continuously observed each day for alterations in eating behaviour, and other unfavourable changes.

For the intracameral injection of the thermo-ocugel, general anaesthesia was induced via intramuscular administration of ketamine HCl, 40 mg/kg (a dissociative aesthetic) and xylazine HCl, 10 mg/kg (a sedative, aesthetic, muscle relaxant, and analgesic) and local anaesthesia induced via instillation of tetracaine HCl 0.5%$^{W}/_{W}$ (topical anaesthetic). The intracameral injection procedure was undertaken by Dr. Aubrey Makgotloe of the Department of Neurosciences, Division of Ophthalmology, University of the Witwatersrand, who is skilled in ocular surgical techniques and possesses the following qualifications: MBBCh, FCOphth, MMed, and in accordance with the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research.

All procedures were conducted in the right eye which was prepared in a sterile manner using a wire lid speculum. The thermo-ocugel was injected into the anterior chamber of the eye. Following the thermo-ocugel injection, a 1% $^{w}/_{w}$ chloramphenicol ointment was immediately administered and thereafter given daily for 3 days to avoid surface ocular infections of the conjunctiva and/or cornea. The rabbits went into recovery mode after the procedure was completed, and post-surgical care was started. This was done by moving the rabbits to a warm room, constantly monitoring for reflexes, respiratory, and heart rate, until the rabbits were fully awake. The rabbits were further observed each day for alterations in eating patterns signifying distress, or any unwanted changes or detrimental effects that could lead to termination of the study. At 24, 48, and 72 hours, the rabbits were assessed visually for any adverse events, that would necessitate the study's discontinuation.

Euthanasia was carried out by an administration of an overdose of IV sodium pentobarbitone (>50mg/kg, ~2mL) at each sampling point i.e.; experimental group: day 3, 7, 14, 21, and 28. Placebo group: day 7, 14, and 28. And negative control: day 0 with consequent enucleation of both eyes and fixing in 10% $^{V}/_{V}$ formalin, followed by embedding in paraffin and staining with haematoxylin-eosin for histomorphological analysis.

4.2.2.3. Histomorphological Analysis for assessment of the degree of ocular inflammation following the intracameral injection of the thermo-ocugel

Groups of rabbits were exposed to the above-described procedures for the *in vivo* intracameral studies, depending on their assigned group, with euthanasia and enucleation at the pre-determined days, in accordance with the scheme presented in Figure 4.1. Rabbits were assigned to the following groups: (n=3 for each group):

- Day 0: Enucleation of control eye (no thermo-ocugel injection) and injection of drug-free hydrogel and thermo-ocugel into rabbit eyes
- Day 3: Euthanasia and enucleation of thermo-ocugel injected eyeballs
- Day 7: Euthanasia and enucleation of drug-free hydrogel and thermo-ocugel injected eyeballs
- Day 14: Euthanasia and enucleation of drug-free hydrogel and thermo-ocugel injected eyeballs
- Day 21: Euthanasia and enucleation of the thermo-ocugel injected eyeballs
- Day 28: Euthanasia and enucleation of drug-free hydrogel and thermo-ocugel injected eyeballs

The enucleated eyeballs were sent to IDEXX laboratories in Pretoria, South Africa, where they were sectioned at three distinct levels along the sagittal axis adjacent to the optic nerve. According to their standard operating procedure, the sections were processed overnight in an automated tissue processor (PTA-his-SOP-27). Wax blocks were made in paraffin wax after regular histological processing, and sections of approximately 6 µm were cut according to IDEXX SOPs (PTA-his-SOP-30). According to the IDEXX SOPs (PTA-his- SOP-49), the slides were stained with haematoxylin and eosin.

Cellular infiltration (degree of lymphocyte, plasma cell, mononuclear, and polymorphonuclear leukocyte infiltration) was graded from 0 to 3+, with Grade 0 indicating no infiltration, Grade 1 indicating mild infiltration, Grade 2 indicating moderate infiltration, and Grade 3 indicating severe infiltration, according to IDEXX laboratory protocols used in related studies [18].

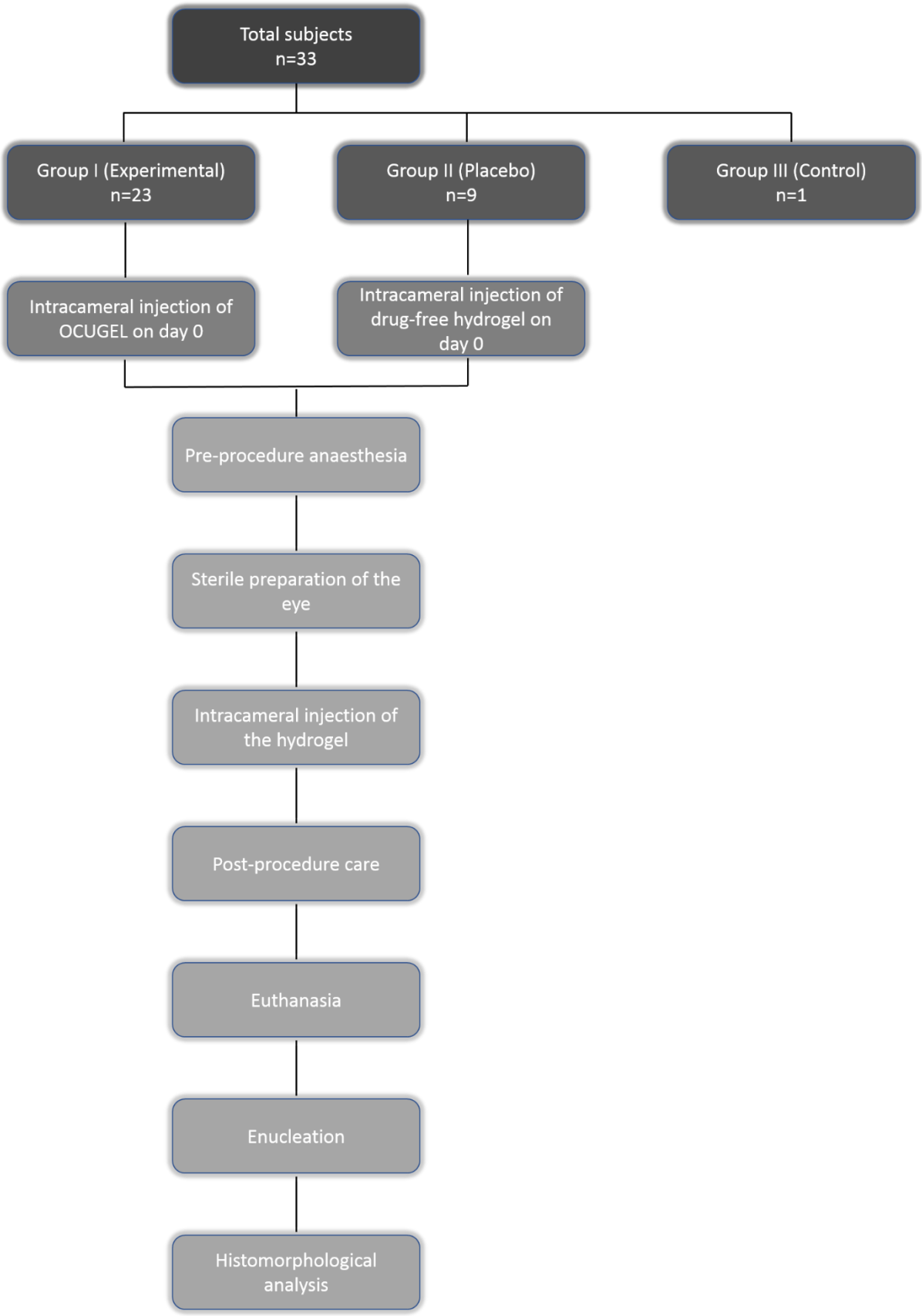

Figure 4.1. *In vivo* study outline (Note: Intracameral injection was undertaken on day 0. Euthanasia and enucleation occurred on days 0, 3, 7, 14, 21, and 28)

4.2.3. Quantification of *in vivo* anti-inflammatory and antibiotic concentrations released from the thermo-ocugel into the ocular fluids of the anterior segment of the rabbit eye model under normal conditions

A high-performance liquid chromatographic (HPLC) method was developed for the simultaneous analysis of MOX and DSP in rabbit anterior ocular fluids using ciprofloxacin as an internal standard. The HPLC was fitted with a C_{18} column. An isocratic method with a run time of 10 minutes was employed using 50:50 acetonitrile and double distilled water as the mobile phase. A solvent flow rate was maintained at 1 mL/min. The extracted and pre-filtered samples (0.2 µL) were injected onto the column. The column and sample temperature were maintained at room temperature (25±0.5ºC). Prior to use, the column was equilibrated by passing the mobile phase solvent through the system. The eluent was monitored at analytical wavelength set at 254nm.

4.2.3.1. Preparation of calibration standards

The preparation of the standards and the samples for drug release followed the method used by du Toit *et al*. [18]. The ocular anterior segment fluid withdrawn from the rabbit eye at predetermined time points (0, 3, 7, 14, 21, and 28) were stored at -80 ºC freezer. The ocular anterior segment fluid withdrawn from the untreated rabbit eye was used as a blank. The frozen blank ocular anterior segment fluids were thawed at room temperature (25±0.5ºC) to equilibrate. The fluids were then centrifuged twice at 15000X for 10 minutes to obtain the supernatant. The isolated and filtered supernatant was further used to prepare stock solution of MOX and DSP (0.2 mg/mL) with standard concentrations ranging between 0.02 to 0.1 mg/mL. To prepare these standard concentrations; 25 µL of each drug was added to 50 µL of the internal standard (ciprofloxacin) followed by addition of 100 µL of the blank ocular anterior segment fluid. The mixture was vortexed for 60 seconds to mix well followed by centrifugation at 15000X for 10 minutes. A total of 300 µL of the mobile phase was added to the mixture followed by further vortexing and centrifugation. An overall volume of 300 µL was filtered through a 0.22 µm filters and transferred into the HPLC injection vials for analysis. The ratio of the peak areas (between the internal standard ciprofloxacin, MOX, and DSP) versus the concentration data for the calibration standards was fit by linear regression to generate the calibration curves (n=3).

4.2.3.2. Sample preparations for drug release analysis

The ocular anterior segment fluids were withdrawn from the thermo-ocugel treated rabbit eye at predetermined time points (0, 3, 7, 14, 21, and 28) were stored at -80 freezer. The frozen

samples were thawed at room temperature (25±0.5ºC) to equilibrate. The fluids were then centrifuged twice at 15000X for 10 minutes to obtain the supernatant. The isolated and filtered supernatant (100 µL) was mixed with the internal standard (50 µL) and vortexed for 60 seconds. A total of 150 µL of the mobile phase was added to the mixture followed by further vortexing and centrifugation. An overall volume of 300 µL was filtered through a 0.22 µm filters and transferred into the HPLC injection vials for analysis. The same procedure used for the calibration standards and HPLC injection was applied for simultaneous measurement of MOX and DSP. All measurements were conducted in triplicate (n=3).

4.3. Results and Discussion

4.3.1. Analysis of the *in vivo* behaviour of the thermo-ocugel in Rabbits

The thermo-ocugel tolerance in rabbit eye models was moderately tolerated. This is demonstrated by its long-term efficacy success. Minimal untoward reactions (e.g. hypopyon, corneal neovascularization) were observed in all the 32 rabbits, some of which demonstrated great resolve overtime when evaluated up to the full 28 days of analysis. This was attributed to the chosen route of administration which may have caused trauma and possibly inadequate sterilization which may have introduced some toxins on to the ocular tissues. However, these reactions did not warrant termination of the study. Furthermore, no rabbit suffered mortality or demonstrated any changes in eating habits before the completion of the study.

Clinically no changes were observed in the choroid, retina, optic nerve, lens, and ciliary body. Mild to moderate inflammation was observed in the cornea and sclera. The general procedure adopted in housing, handling, anaesthesia, and post-surgical care of the rabbit is depicted in Figure 4.2.

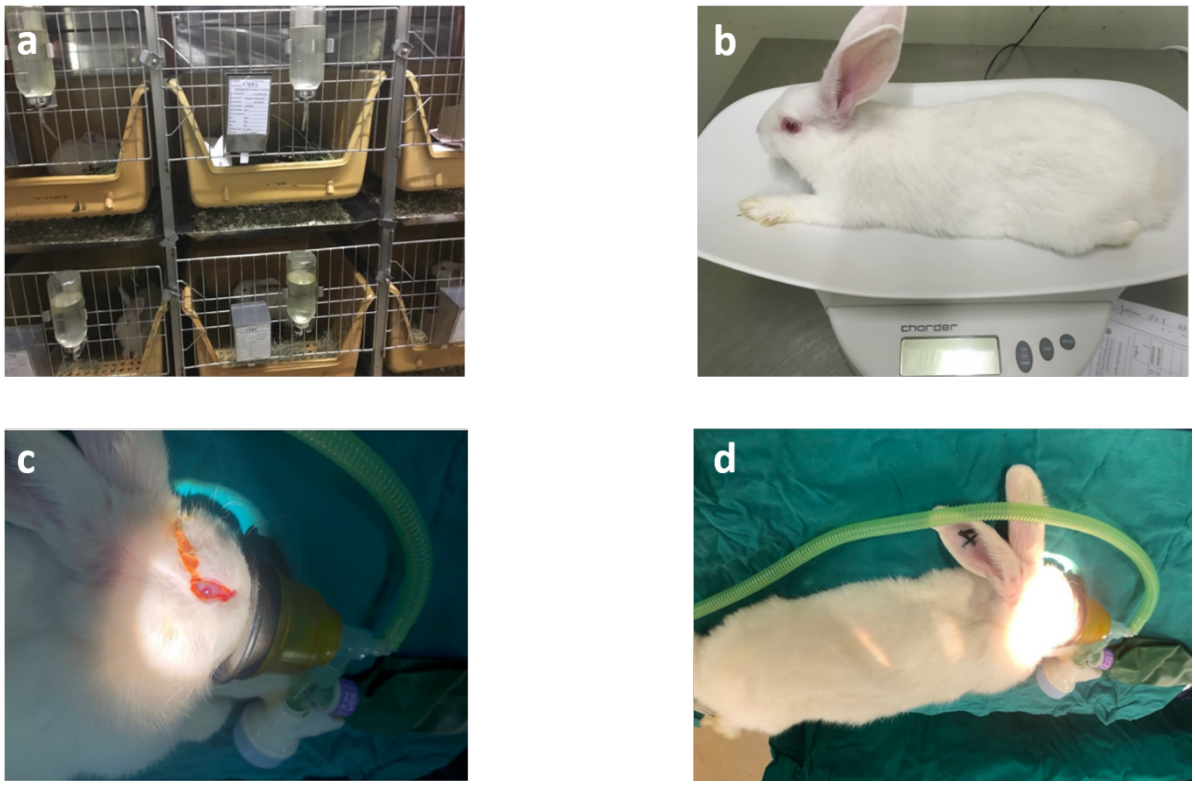

Figure 4.2. Photographic documentation of (a) rabbit housing (b) weighing (c) preparation for surgery with anesthetization (d) post-surgical care

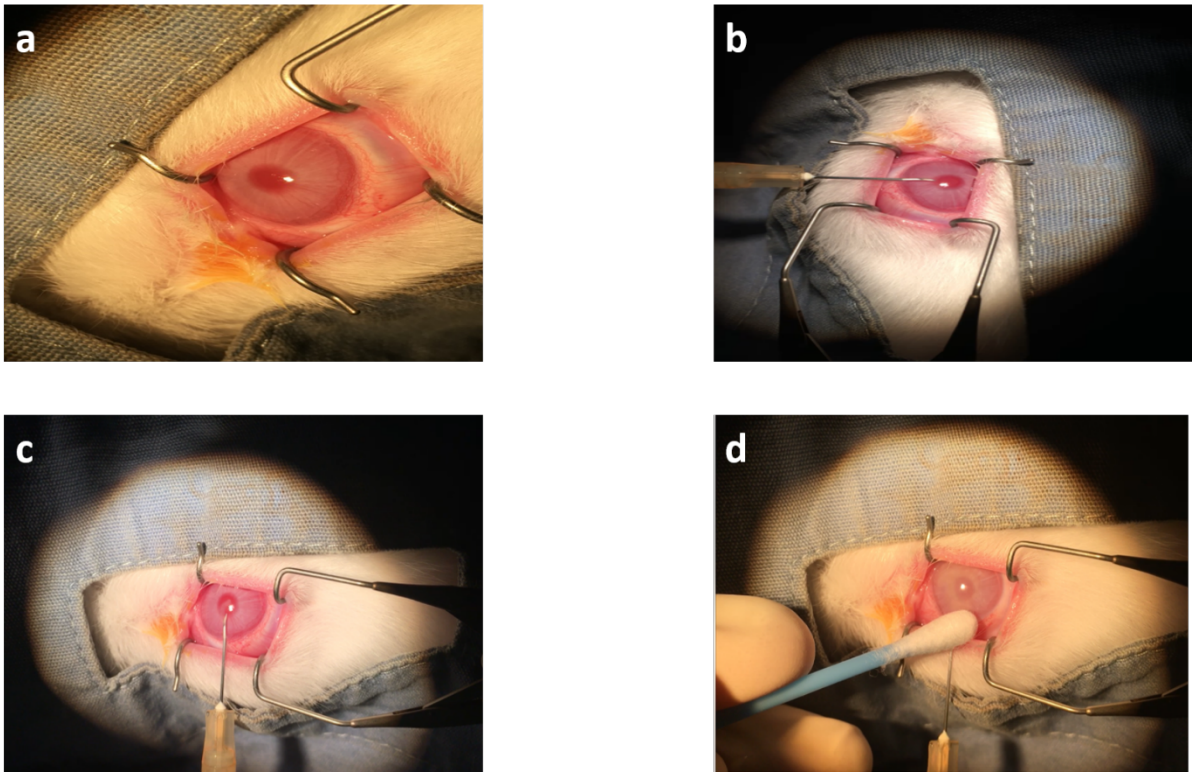

Figure 4.3. Images (a-d) depicting consecutive stages in the intracameral injection of the thermo-ocugel into the anterior segment of the rabbit eye

4.3.2. Histomorphological findings following intracameral injection of the thermo-ocugel and drug-free hydrogel in normal rabbit eye

Histomorphological evaluation of the ocular milieu safety and biocompatibility was undertaken on all representative samples (i.e.; experimental, placebo, and control). Figure 4.4 depicts sagittal slices from the rabbit eye where no hydrogel was injected and serves as a point of reference, and showed normal morphology with no inflammation seen in both the anterior and posterior chambers.

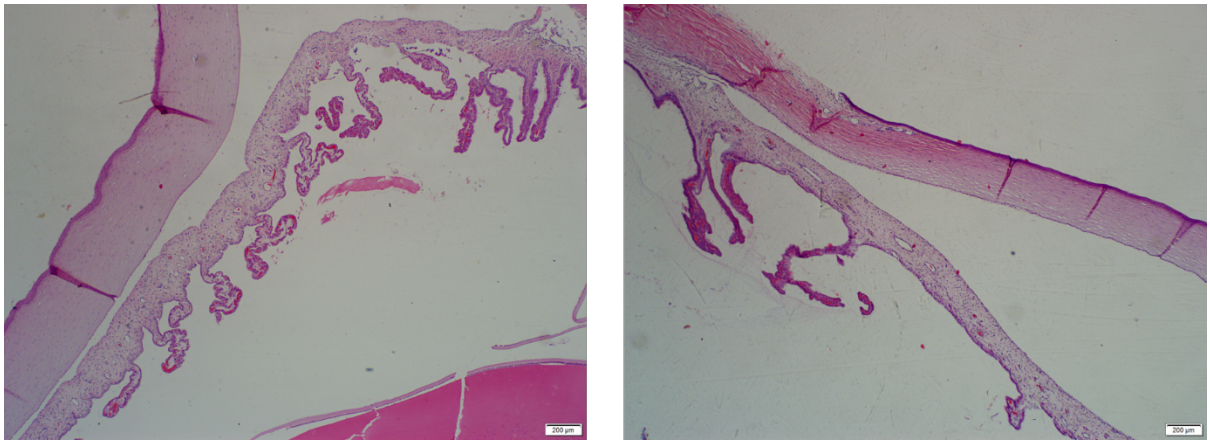

Figure 4.4. Images of the normal eye in the control rabbit which depicts normal appearance of the structures of the globe at 4x magnification

4.3.2.1. Effects of the thermo-ocugel in the normal rabbit eye at day 3

Three days following the intracameral injection of the thermo-ocugel, an evidence of moderate-severe fibrinopurulent exudate was observed in the anterior chamber of the eye, but mostly attached to the anterior surface of the iris. This may have been a reaction instigated by the introduction of foreign bodies into the ocular tissues. The overlying cornea did not show specific changes but at the limbus, mild fibroplasia and haemorrhage was visible accompanied by few infiltrating heterophils and single lymphoplasmacytic cells. Multifocal small aggregates of heterophils invaded the iris and ciliary body stroma with mild capillary congestion. The lens appeared normal, and no specific changes were visible in the retina and optic nerve (Figure 4.5).

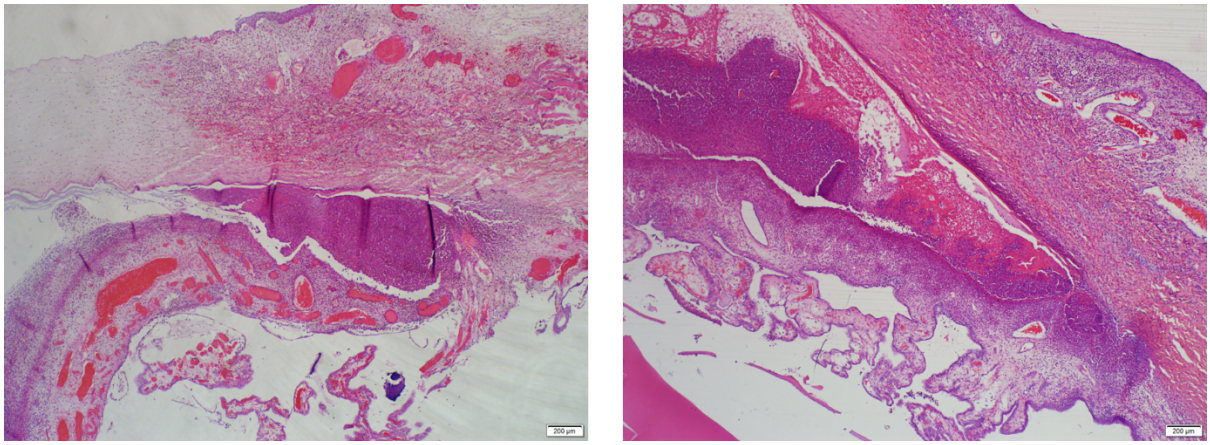

Figure 4.5. Histological slides at day 3 for the thermo-ocugel in the normal eye at 4x magnification

4.3.2.2. Effects of the drug-free hydrogel and thermo-ocugel in the normal rabbit eye at day 7

At day 7, morphological evaluation of sections from the eyeball showed confirmed cases of severe fibrinopurulent exudate in the anterior chamber of the eye for both the drug-free hydrogel and thermo-ocugel. A few inflammatory cells are also visible in the posterior chamber for the drug-free samples which extended to the lens surface. However, the posterior segment for the thermo-ocugel samples did not show any indication of inflammatory cells. The iris and ciliary body appeared to be mildly to moderately oedematous within the stroma accompanied by extensive infiltration of heterophils and few lymphoplasmacytic cells. Moderate numbers of heterophils infiltrated the stroma at the limbus and capillaries appeared mildly dilated. The lesion extended along the peripheral corneal substantia propria. Specific lesions were not visible in the lens and the retina appeared normal (Figure 4.6a).

The morphological changes observed above were similar to those observed on the thermo-ocugel samples. However, the thermo-ocugel samples exhibited moderate oedema with mild heterophilic invasion within the stroma and mild presence of heterophils and mononuclear leukocytes including lymphoplasmacytic cells and macrophages in the ciliary body. The limbus had mild presence of heterophils interstitially and multifocal minimal haemorrhage. Similar to drug-free samples, specific changes were not visible in the lens and retina. The optic nerve appeared normal, but the surrounding encasing connective tissue contained few perivascular lymphocytes (Figure 4.6b).

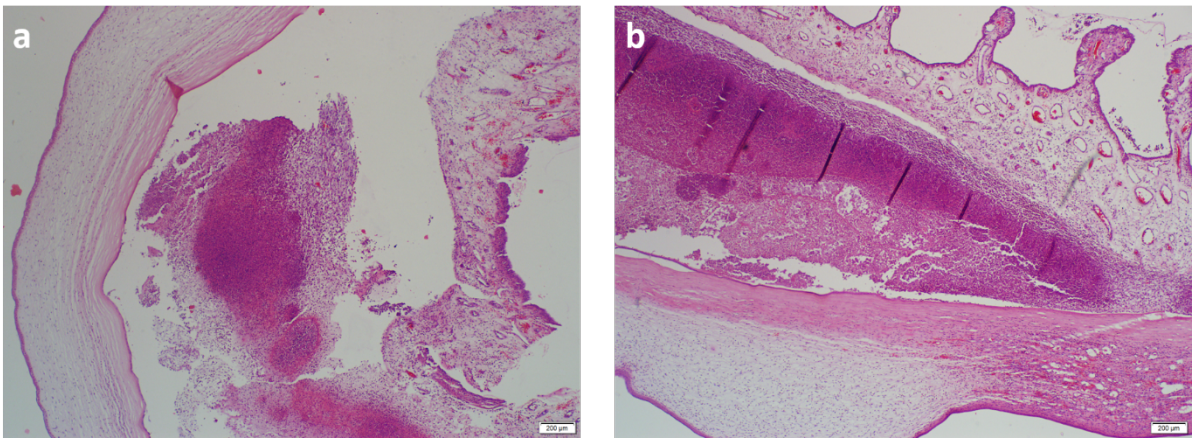

Figure 4.6. Histological slides at day 7 for the drug-free hydrogel (a) and thermo-ocugel (b) in the normal eye at 4x magnification

4.3.2.3. Effects of the drug-free hydrogel and thermo-ocugel in the normal rabbit eye at day 14

At day 14 of the experiment, the eyeball sections for drug-free hydrogel-injected specimens showed notable presence of fibrinopurulent exudate within the anterior chamber. The exudate centrally consisted of degenerate heterophils and fibrin. The exudate was slightly visible towards the posterior region and peripherally attached to the iris with prominent fibroplasia and horizontally arranged fibroblasts. The inflammation extended into the limbus, involving the stromal tissues unilaterally. The peripheral iris and root of the ciliary body showed oedema and moderate heterophilic invasion. The corneal substantia propria showed moderate inflammation by heterophils as well as multifocal aggregates of lymphocytes. Specific lesions were not visible in the lens, retina, optic nerve and choroid (Figure 4.7a).

The thermo-ocugel injected eyeball specimens exhibited similar morphology to that of drug-free injected eyeballs, although the eyeball sections showed a lower infiltration of heterophils admixed with fibrin in the anterior chamber. The fibrinopurulent exudate showed partial attachment to the anterior surface of the iris with small to moderate numbers of macrophages lymphocytes and plasma cells are present. There was mild infiltration of similar cells into the iris stroma. The limbus yielded moderate invasion of heterophils and few mononuclear leukocytes into the peripheral stroma while the rest of the substantia propria of the cornea showed mild oedema and heterophilic invasion. The ciliary body, lens, retina, choroid and optic nerve appeared normal and did not show specific lesions (Figure 4.7b). Furthermore, there was a prominent fibroblast presence in the thermo-ocugel injected samples in comparison to the drug-free injected samples which infers a greater extent of tissue regeneration purportedly instigated by the presence of the anti-inflammatory agent.

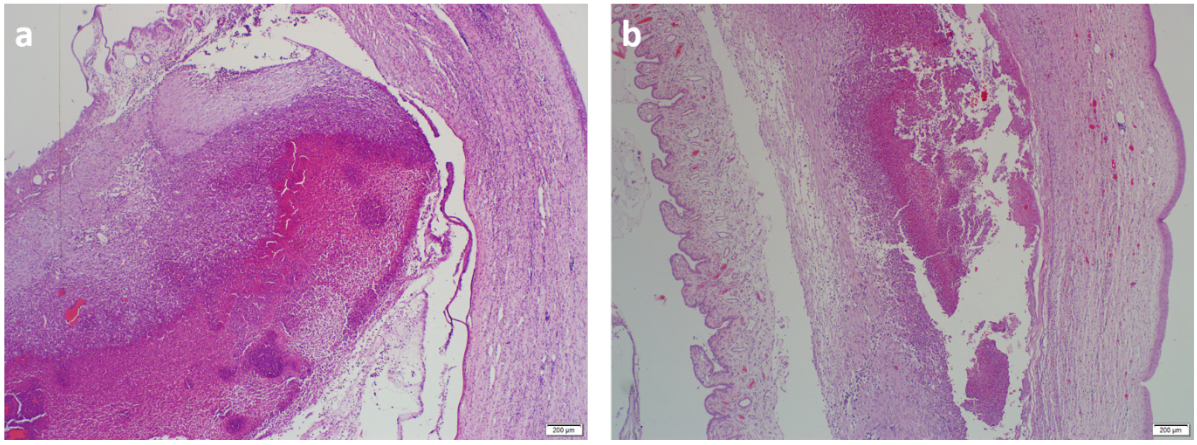

Figure 4.7. Histological slides at day 14 for the drug-free hydrogel (a) and thermo-ocugel (b) in the normal eye at 4x magnification

4.3.2.4. Effects of the thermo-ocugel in the normal rabbit eye at day 21

To confirm the findings purporting to ocular tissue regeneration capabilities for the thermo-ocugel injected samples, the histological evaluation was further carried out on day 21 of the experiment. The morphological evaluations confirmed optimal tissue regeneration where the analysed specimens demonstrated insubstantial histological changes in comparison to the control. There was minimal to no exudate within the anterior chamber of the eyeball at day 21. This further substantiate the wound healing performance of the thermo-ocugel. The corneal substantia propria revealed mild cellularity due to very mild fibroplasia. The ciliary body and posterior iris showed prominent processes and folding which may be reactive, but not associated with inflammation or oedema. No other changes were visible (Figure 4.8).

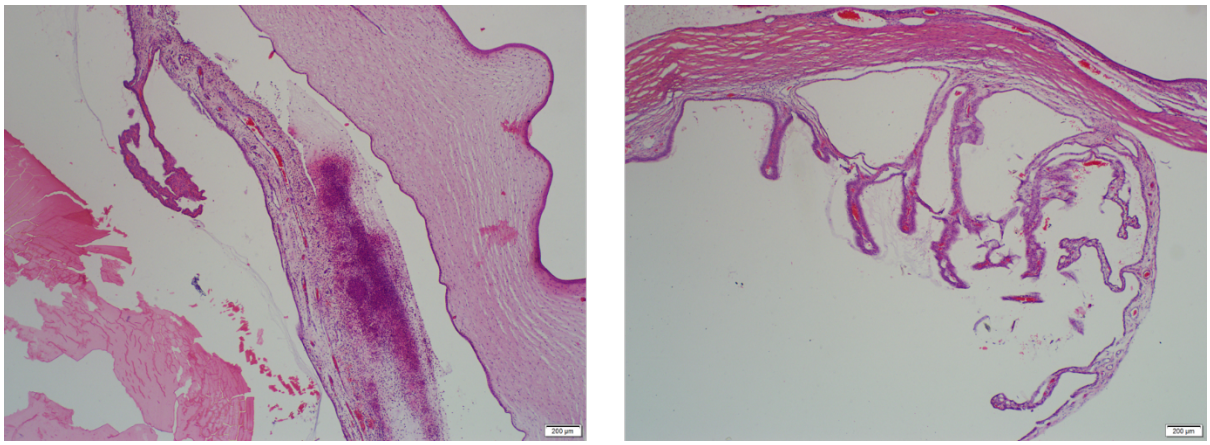

Figure 4.8. Histological slides at day 21 for the thermo-ocugel in the normal eye at 4x magnification

4.3.2.5. Effects of the drug-free hydrogel and thermo-ocugel in the normal rabbit eye at day 28

At day 28, histological evaluation of the sections from the drug-free hydrogel-injected eye demonstrated severe cases of fibrinopurulent exudate in the anterior chamber of the eye. The exudate was attached to the posterior surface of the cornea. The Descemet's membrane was thickened, hyalinised and detached in some areas. Heterophils invaded the posterior corneal substantia propria and were focally associated with a large area of calcification in the proprial stroma. There was occasional presence of degenerate heterophils and fibrin close to the lens surface, associated with single vacuolated macrophages (Figure 4.9a).

Histomorphological evaluation of the sections from the thermo-ocugel injected eye demonstrated no sign of fibrinopurulent exudate in the anterior chamber. The corneal substantia propria contained a few fibroblasts especially more anteriorly. The corneal clearing is indicative of no permanent damage and functional restoration of ocular tissues [19, 20]. In addition, slit-lamp examination also confirmed the resolution of anterior segment inflammation and corneal oedema which further ruled out the possibility of permanent damage such as chronic inflammation, cystoid macular oedema, and secondary glaucoma due to severe trabecular meshwork damage [21]. The posterior segment did not appear to be affected. The iris stroma also appeared normal with no prominent inflammation (Figure 4.9b).

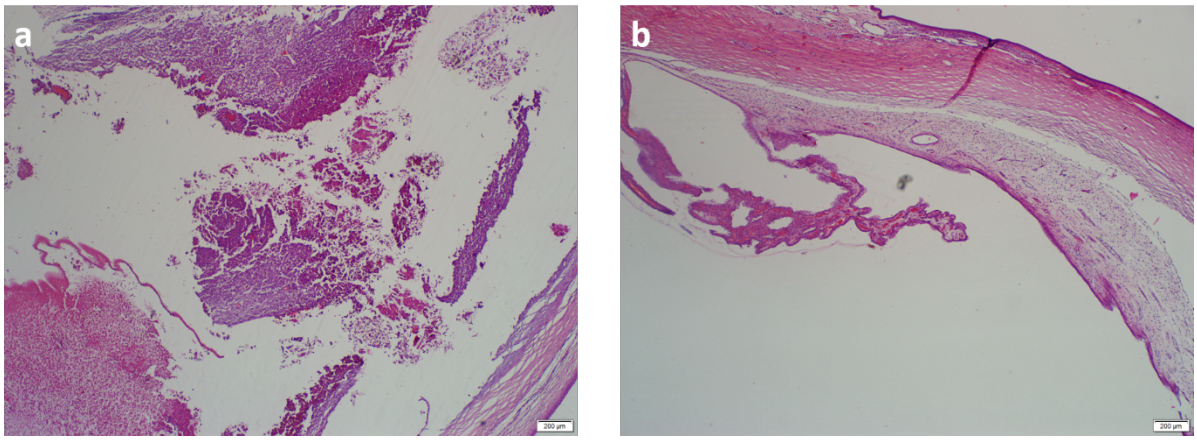

Figure 4.9. Histological slides at day 28 for the drug-free hydrogel (a) and thermo-ocugel (b) in the normal eye at 4x magnification

4.3.3. Overall effect of the drug-free hydrogel and the thermo-ocugel in the eye

The *in vivo* biocompatibility and ocular tissue response assessments provided insights on the tissue remodelling performance of the hydrogels at different stages. The morphological findings in several sections where the thermo-ocugel and drug-free hydrogel were left for 3, 7, and 14 days in the anterior chamber of the normal rabbit eye showed moderate fibrinopurulent exudate which consists of heterophils and fibrins. This could have been instigated by the selected route of administration which is associated with TASS-induced inflammation [22]. Mild inflammation is also observed on the anterior surface of the iris and the stroma in some rabbit eyes. The inflammation is confirmed by mild presence of heterophils and mononuclear leukocytes including lymphoplasmacytic cells and macrophages. The intracameral injection is also considered an injury thus some of the inflammatory reaction may have been due to the injection. Minimal haemorrhages were detected around the limbus of the eye in a few cases. These could be related to the inflammatory reaction or an unintended consequence of the final enucleation of the eye for histological evaluation. At day 14, the presence of fibroblasts is observed on specimens. The fibroblast reactions were indicative of the initiation of tissue regeneration [23]. The difference in the fibroblast presence amongst the day 14 specimens was that it was prominent in the thermo-ocugel injected samples than the drug-free injected samples. The occurrence of the fibroblastic reaction further infers that there was a greater extent of tissue regeneration in the thermo-ocugel injected samples.

In the more long-standing samples (i.e. days 21 and 28), the morphological evaluation for the thermo-ocugel injected eyes revealed only a few proliferating fibroblasts in the anterior chamber and with no exudate. This highlights a significant reduction in level of inflammation which could be attributed to adequate release of the corticosteroid (DSP) loaded in the thermo-ocugel. The drug-free specimens for day 28 confirmed the presence of a fibrinopurulent exudate in the anterior chamber. This is due to the absence of an anti-inflammatory drug to aid in the reduction of the inflammation as opposed to where a drug-loaded hydrogel was injected. This ultimately proves the efficacy of the thermo-ocugel in counteracting the pathological tissue changes that occur when inflammation is prompted (Figure 4.10). although inflammation was instigated following intracameral injection of the hydrogels, the introduction of the antibiotic and anti-inflammatory agents at the point of cataract surgery to counteract the post-surgery sequelae such as postoperative endophthalmitis, outweighs the inflammation instigated by the initial injection and foreign body reaction instigated following the intracameral injection. Furthermore, although the drug-free hydrogel-injected ocular samples did not demonstrate notable tissue regeneration, no notable or permanent damage to ocular structures was observed, highlighting the adequate safety of the hydrogel system.

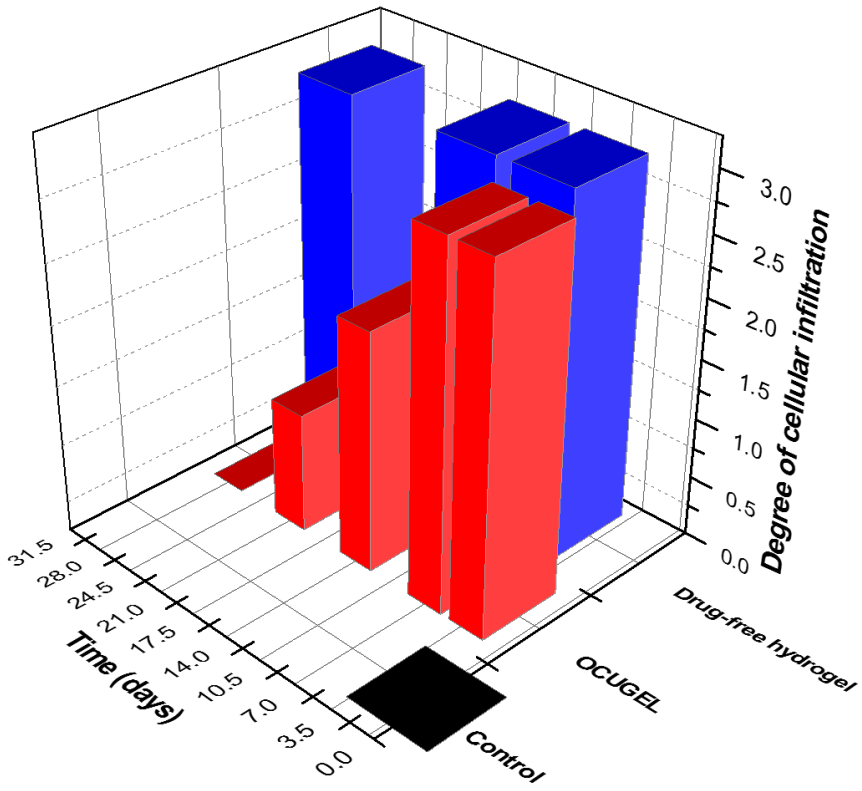

Figure 4.10. Graphical representation depicting the degree of cellular infiltration by lymphocytes, plasma cells, and mononuclear and polymorpho-nuclear leukocytes over time for control, thermo-ocugel and drug-free hydrogel injected eyeball samples

4.3.4. Quantification of *in vivo* anti-inflammatory and antibiotic concentrations released from the thermo-ocugel into the ocular fluids of the anterior segment of the rabbit eye model under normal conditions

The standard curves of MOX and DSP were obtained by measuring the area under the curve of each drug in the presence of ciprofloxacin as the internal standard. The chromatographic analysis of ciprofloxacin, MOX and DSP were measured at 254 nm with their respective retention times at 0.820, 1.126, and 3.057, respectively. Calibration curves generated for both MOX and DSP are depicted by Figure 4.11a & b. The release profiles of MOX and DSP were derived from the calibration curves and represented in Figure 4.11c & d. The therapeutic effect of MOX and DSP were reported to be from 0.2 µg/mL and 0.5164 µg/mL , respectively [24, 25]. The drug release profile of MOX and DSP were higher than the minimum effective concentrations (MEC) for the recommended durations. Literature reports indicated that concentrations of MOX up to 150 mg/mL and 500 mg/mL had no toxic effect in corneal endothelial cells from 24 hours to 28 days, respectively [26]. The release profile of MOX in the

current study highlighted that the highest concentration (0.62 ± 0.29 mg/mL) was attained at day 3, with levels dropping just below the MEC by day 7 and decreasing in concentration up to 0.11 ± 0.06 mg/mL by day 28 (Figure 4.11c). The high therapeutic concentrations (>0.2 µg/mL) attained in the first 7 days highlight that there would be sufficient MOX concentrations to induce a therapeutic antibiotic effect while still remaining under the reported non-toxic concentrations of 150 µg/mL for the required antibiotic treatment period of 7 days. The current treatment requires that patients administer MOX eye drops 4 times a day for 7 days [27]. The frequent administration of these eye drops has been associated with poor patient compliance which in turn reduces positive treatment outcomes [28]. The release of DSP on the other hand displayed an increasing concentration profile with levels greater than 2.16 ± 4. 62µg/mL from day 3 (Figure 4.11d). Sustained levels of DSP were maintained above the MEC (0.5164 µg/mL) for the 28-day period of investigation. Although the DSP concentrations are above the MEC, they were still below the reported minimum toxic concentration of 1 mg/mL which infers that levels were sufficient to induce therapeutic effect without inducing any toxicity [24]. For the current treatment of inflammation, the success of post-cataract surgical care requires the administration of 2 eyedrops daily for 30 days, which may not be ideal for most patients such as minors and the elders who may be dependent on the assistance of caregivers [27]. The thermo-ocugel thus achieves therapeutic levels of both the antibiotic and anti-inflammatory agent over the required treatment periods, while significantly reducing administration frequency.

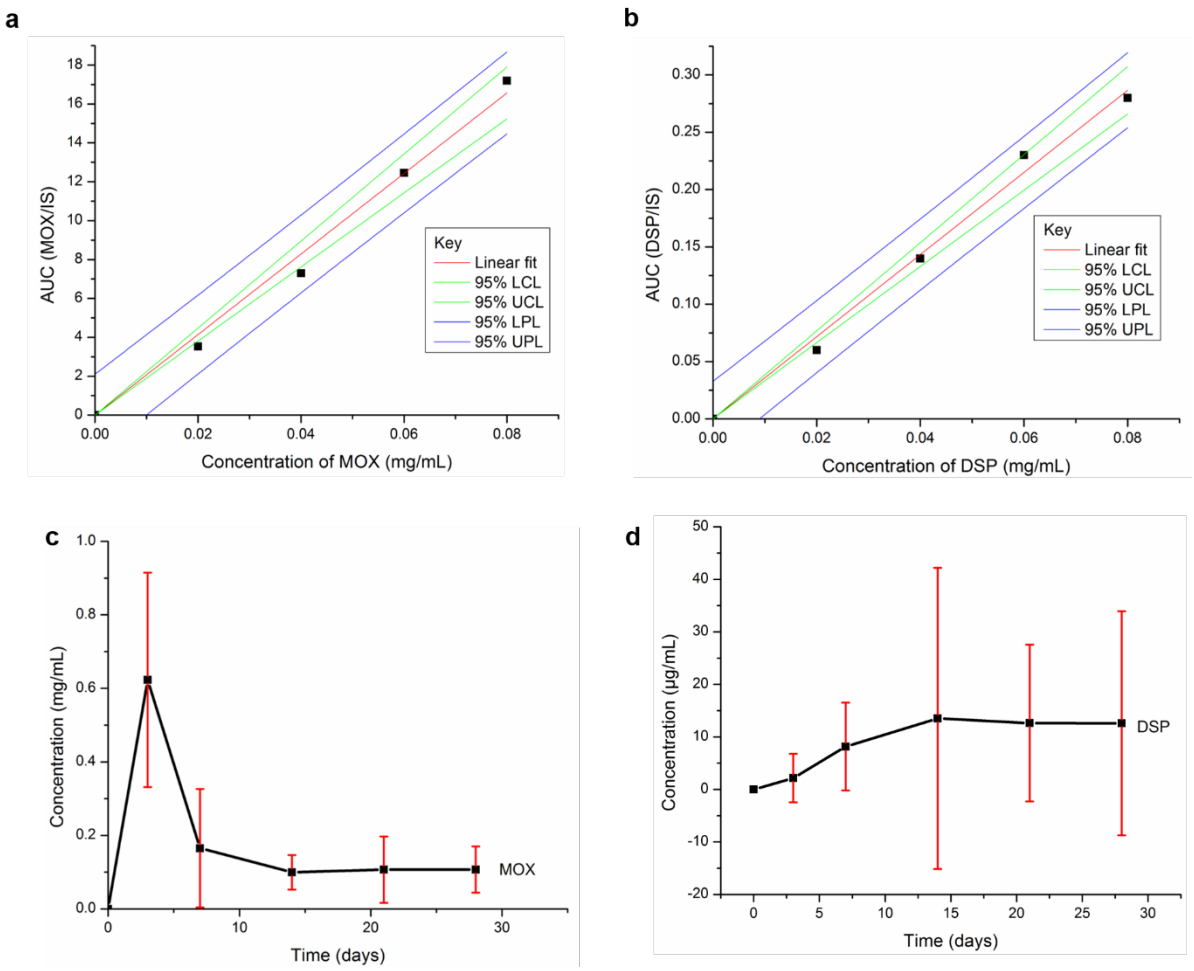

Figure 4.11. Calibration curves derived for (a) MOX and (b) DSP in the presence of ciprofloxacin as an internal standard, in ocular fluids of the anterior segment. *In vivo* drug release profiles of (c) MOX and (d) DSP in normal rabbit eye.

4.4. Concluding Remarks

The morphological findings following the intracameral injection of the hydrogels indicated cases of moderate-severe fibrinopurulent exudate in the anterior chamber. This was as a result of the introduction of the hydrogels - which are perceived as foreign bodies - into ocular tissues. Moreover, inflammation occurred as a result of the injury relating to the intracameral injection. The inflammation resolution was evident on the third week (day 21) as a response to the steroid loaded into the thermo-ocugel, whereas less signs of clearance or reduction of inflammation was observed on any of the drug-free specimens at this period. The DSP-loaded thermo-ocugel offered prompt control of inflammation which is essential in the prevention of any permanent damage such as persistent corneal oedema, chronic persistent inflammation, and cystoid macular oedema or irreversible harm to delicate ocular structures such as the corneal endothelium, trabecular meshwork and macula, which could occur following cataract

surger, as well as for the prevention of more devastating cataract post-surgical effects such as POE. The inflammation resolution is further indicative of adequate *in vivo* drug concentrations which is a good preliminary indication that the thermo-ocugel was effective in counteracting the pathological tissue changes that occur when inflammation is prompted. Furthermore the *in vivo* drug release of the two drugs indicated concentrations which were well-maintained within the therapeutic window for both drugs. The thermo-ocugel achieved therapeutic levels of both the antibiotic and anti-inflammatory agent over the required treatment periods, while significantly reducing administration frequency. Considerations for future investigations could focus on inducing cataract in the animal model followed by surgery and treatment by thermo-ocugel to assess anti-inflammatory and anti-bacterial properties.

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CHAPTER 5

Conclusion and Recommendations

5.1. Conclusion

There has been great advancement in the ocular drug delivery field. The introduction of novel polymeric systems with bioresponsive properties has gained considerable amount of attention in this field mainly for successful direct and controlled delivery of ocular therapeutics. However, there is still no drug delivery system that meet the requirements for an ideal ocular drug delivery system. The study's main goal was to incorporate these findings into the design and fabrication of an *in situ* forming thermoresponsive ocular hydrogel as a drug delivery system. The fabrication of an *in situ* forming system as a novel technique for delivering therapeutic drugs like antibiotics and anti-inflammatories for the proposed enhanced treatment of post-operative infections and inflammations resulted from this work. Herein, the sol-gel transitional states were fully manipulated in the development and delivery of the fabricated hydrogel systems. The double crosslinking approach was used to fabricate these systems and further allowed for dual drug incorporation of moxifloxacin HCL and dexamethasone sodium phosphate. The use of biodegradable biomaterials such as protein-derived polymers (gelatin) and polysaccharides (chitosan) aided the biocompatibility and degradation of this ocular drug delivery system.

BGP was used for its catalystic behaviour for the triggering of a solution-gel transition in CS solutions at ocular physiological conditions i.e.; pH and temperature. The sol-gel transition is initiated by the mechanism for glycerol phosphate crosslinked chitosan occurs by ionic interaction between the quaternary ammonium groups of chitosan and the phosphate moiety of BGP [1]. An increase in the formation of hydrogen bonds and further gel formation acceleration resulted from the addition of gelatin. This is a contribution of the interaction of hydroxyl and amino groups of chitosan and the hydroxyl groups of gelatin [2]. These hydrogels exhibited the desired physico-mechanical properties, but the achievement of a 28 day dual drug release remained impossible. Thus, further modification of the hydrogels was needed for attainment of prolonged dual drug release profile. To achieve this, the CSBGPGEL hydrogel solution was crosslinked with genipin. Genipin reacts promptly with chitosan, as well as with proteins such as gelatin or amines in general, thus its use resulted in a more structurally rigid hydrogel (thermo-ocugel) with feasible rheological properties capable of incorporating moxifloxacin HCL and dexamethasone sodium phosphate with the encapsulation efficiency

75.27% and 91.48% as well as drug loading of 6.14% and 1.87% for MOX and DSP, respectively

The thermo-ocugel exhibited excellent thermostability with the melting point at 150 °C and degradation occurring at 338 °C. The thermo-ocugel further displayed desirable visco-elastic properties characterized by overtime increase of storage modulus and the loss modulus. The sol state to sol-gel state was characterized by a slight increase in loss tangent. The inverse correlation between the increased viscosity and rigidity of the hydrogel against the sample height implies that as the gel forms the hydrogel it became physically rearranged to form a stable and stiffer contracted gel. To confirm the *in situ* forming thermoresponsitivity, the thermo-ocugel displayed a gel point at the temperature height of 32 ^{o}C which is a great indication that the thermo-ocugel will be a gel at ocular physiological temperature and in turn show great capability of retaining the drugs for a prolonged period. This was further supported by the *in vitro* drug release results.

The thermo-ocugel showed a burst release of 52.75 ± 3.12% and 60.94 ± 8.31% in 12 hours which increased to 76.61 ± 5.91% and 81.71± 4.85% in 3 days followed by a steady release of 0.53 ± 0.15 % and 0.81 ± 0.26% per week for DSP and MOX, respectively. The high burst release occurred during the sol-gel state transition and this stage is crucial in the early stages of post-operative care. In addition, the high burst release (>50%) and the sustained release of <1% per week indicates that the incorporated MOX and DSP could exert notable therapeutic effects at the early stages of wound healing and also present sufficient concentrations to maintain the effective therapeutic levels for the required durations for each respective drug, which is of essence in a successful treatment of post-cataract surgery infection and inflammation. Further *in vitro* studies conducted on the thermo-ocugel at physiological conditions revealed that the swelling-cumulative release behaviour exhibited a biphasic relationship with the initial phase mimicking a first order relationship observed in the first 3 days followed by a zero order relation from 3 days to 28 days. The first-order relationship infers that most of the drug is released in the first 3 days with slight degradation and zero-order is coupled with minimal drug release, which was observed when the surface erosion increased.

These findings provided a prediction of and correlation with the *in vivo* performance of the thermo-ocugel where the potency of the developed thermo-ocugel was successfully demonstrated by its ability to maintain both antibiotic and anti-inflammatory drug concentrations within their acceptable therapeutic ranges thereby resolving most of the inflammation in 3-4 weeks.

5.2. Recommendations

A novel fabrication approach was explored in designing a dual drug loaded thermoresponsive chitosan-gelatin hydrogel with optimal properties translating to effective performances such as *in vivo* compatibility, and wound healing performances. This was undertaken in order to corroborate the feasibility and clinical viability of the intracamerally injected thermo-ocugel in human patients. The double crosslinking approach was employed in designing the *in situ* forming thermoresponsive hydrogel. Properties such as thermo-physical properties, fluid uptake, rheological properties (elasticity/rigidity, viscosity and sol/gel state), and degradation were improved to suit performances such as dual drug loading, biocompatibility and wound healing.

In this study, the thermo-ocugel was fabricated with the intention to have it function as an ocular drug delivery system engineered for the potential treatment of infection and inflammation. The ability of the fabricated thermo-ocugel to show desired and optimum response to simulated ocular conditions served as a stepping-stone and indicator that it will operate in a more tailored, patient-specific manner to offer effective therapy to patients on an individual basis. The exploitation of pharmaceutical technologies in developing systems with desirable mechanical properties such as in the current study has the potential to advance ocular drug delivery and post-operative care. The superior properties conferred through design of bioresponsive systems by employment of natural polymers and crosslinking approaches would enable the development of versatile delivery system that could be used as platform for the site-specific delivery of a potentially diverse array of therapeutics to various tissue sites [5, 6]. With continuous research and review of the pitfalls of these novel ocular systems, new trends in ocular drug delivery are enforced, each with substantial contribution to the field and the potential for huge health and economic benefits.

Further recommendations to progress this present study to final development and marketing include the exploration of alternative ocular routes of administration as a necessary measure to avoid triggering injury-induced inflammation [3, 4], as well as expanded preclinical studies in a cataract model.

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Appendix A: Animal Ethics Clearance Certificate

ANIMALS RESEARCH ETHICS COMMITTEE (AREC)

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

STRICTLY CONFIDENTIAL

CLEARANCE CERTIFICATE NUMBER: 2020/03/05/C

APPLICANT: Ms N J Lekoloane
School: School of Therapeutic Sciences; Department: Pharmacy and Pharmacology; Location: CAS

PROJECT TITLE: Bioresponsive slowly degrading, polymer dual drug conjugate as an injectable system for ocular anterior segment infection and inflammation intervention in New Zealand white rabbits;
Category: C; Species and Numbers involved: *33X 1.5 - 2.5kgs male or female New Zealand White Rabbits*

Approval is hereby given for the use of animals for the research project named above and described in the application reviewed by a quorate meeting of the AREC held on 31 Mar 2020. This approval remains valid until 21 Sep 2022 and is conditional to the following (if blank there are no special conditions):

Condition 1	Condition 2	Condition 3	Condition 4

All material changes to the approved research must be reported to the AREC before they are implemented. Failure to do so will invalidate this clearance certificate.

An annual progress report must be provided to the AREC.

The use of these animals is subject to AREC guidelines on the use and care of laboratory animals, is limited to the procedures described in the application and is subject to additional conditions listed below:

I, the Chair of the AREC (or my designated representative) am satisfied that the proposed research is ethical as judged by local law, international standards and University policy.

Signed: _____ _____ Date: _28/09/2020___
(Chairperson of the AREC)

I am satisfied that the persons listed in this application are competent to perform the procedures described in the application, in the context of Section 23 (1) (c) of the veterinary and Para-veterinary Professions Act (19 of 1982).

CC: Student supervisor: «Title1» «Initials1» «Supervisor_surname»
Director Central Animals Service: Dr Kim Jardine

Appendix B: Review Paper

Thermosensitive Injectable Hydrogels as Ocular Drug Delivery Systems for Postoperative Endophthalmitis

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Abstract

Post-operative endophthalmitis (POE) following ocular surgery is a vision-threatening complication. Prompt administration of antibiotics and corticosteroids is critical following surgery as a prophylactic measure against POE. Accordingly, studies have investigated and successfully identified antibiotics and corticosteroids which possess the potency for potential use in POE prophylaxis. The past decade has seen a substantial rise in the emergence of *in situ* gelling thermosensitive hydrogels due to their potential for provision of a more effective and reliable progression in achieving more practical, tailored, and patient-specific therapies. One of the concerns in ocular formulations is the lack of prolonged drug release which lead to frequent formulation administration to prevent secondary infections. However, there is still no single antibiotic or corticosteroid that covers efficiently all the organisms that induce postoperative endophthalmitis especially in cases where inflammation is present. Therefore, a combination of at least two drugs (ie; antibiotic and steroid) is thus required. The quest for designing novel ocular drug delivery systems has gained significant attention in recent years to improve versatility and capability in incorporating hydrophilic/lipophilic antibiotics and corticosteroid for a prolonged release. This review evaluates the employment of *in situ* gelling thermosensitive hydrogels systems carrying antibiotics and corticosteroids for POE prophylaxis. This will give insights on concise perspective for prospective trends in POE prophylaxis.

Key words: thermoresponive, hydrogels, antibiotics, corticosteroids, ocular, Post-operative endophthalmitis

Fabrication of a dual drug loaded thermoresponsive chitosan-gelatin hydrogel for potential treatment of post-cataract surgery infection and inflammation

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Abstract

Hydrogels for ocular drug delivery offer numerous advantages over conventional ocular drug delivery systems such as controlled release of the loaded drug, increased drug retention time and improved biocompatibility. The current study investigated the development of dual-crosslinked chitosan-gelatin hydrogel ('Ocugel') for potential application in the delivery of drugs to the anterior segment of the eye. The Ocugel, which comprised of chitosan (CS), gelatin (GEL), β-Glycerophosphate (BGP), genipin (GEN), moxifloxacin HCL (MOX), dexamethasone sodium phosphate (DSP), was developed for intracameral administration. The Ocugel exhibited thermoresponsive behaviour with the gel formation occurring within a temperature range of 25 to 38 °C with the highest gelling point at 32 °C. Rigidity (G') of the Ocugel started increasing from 5 hours of genipin crosslinking and reached 3.5 kPa in 12 hours. The Ocugel allowed for dual drug encapsulation efficiencies of 91.48% and 75.27%, and drug loading of 1.87% and 6.14% for DSP and MOX, respectively. The Ocugel showed a burst release of 52.75 ± 3.12%, and 60.94 ± 8.31% in 12 hours, which increased to 76.61 ± 5.91% and 81.71± 4.85% in 3 days, followed by a steady release of 0.53 ± 0.15 % and 0.81 ± 0.26% per week for DSP and MOX, respectively. The Ocugel showed a rapid fluid uptake of 126.02% in the first 3 days followed by a gradual increase up to 198.72% in 28 days. The Ocugel exhibited a slow erosion (1.5%) on the first 3 days whereas a slightly higher degree of erosion rate (16.58%) was observed between 3 and 14 days and the overall surface erosion was 18.20% in 28 days. The dual drug incorporation, fluid uptake and surface erosion properties of the fabricated novel Ocugel were suitable for the application as potential treatment of post-cataract surgery infection and inflammation.

Keywords: Ocugel, Intracameral, crosslinking, ocular, dual drug loading, thermoresponsive.

Appendix D: Research Paper 2

In vivo studies conducted on dual drug loaded thermoresponsive chitosan-gelatin hydrogel (OCUGEL) for potential treatment of post-cataract surgery infection and inflammation

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Abstract

Thermoresponsive hydrogels have drown much attention in the field of ocular drug delivery due to the use of biocompatible and biodegradable biomaterials such as protein-derived polymers (gelatin) and polysaccharides (chitosan). The use of these biomaterials offers more tailored, patient-specific features that aid in the provision of optimal tissue regeneration and wound healing. A previous study introduced a novel approach in designing and fabricating moxifloxacin HCL and dexamethasone sodium phosphate loaded *in situ* forming chitosan-gelatin hydrogel (OCUGEL) with favourable physicochemical properties, optimal mechanical properties, and dual drug incorporation. Herein, the fabricated hydrogel was investigated for possible translational performances such as *in vivo* compatibility and ocular tissue response to the introduction of foreign bodies (hydrogels). Morphological findings of the sectioned eyeballs indicated moderate-severe reactions on the first 14 days of introducing of the hydrogels into the anterior segment. However, the lens, retina, optic nerve and choroid, which form part of the posterior segment, were not affected at all, they remained normal. The severe reactions in the anterior segment included the presence of fibrinopurulent exudate and inflammation in the anterior surface of the iris and the stroma in some rabbit eye. This could have been instigated by the selected route of administration which has been reported to perpetuate devastating inflammation. The morphological evaluation of the OCUGEL injected eyes revealed only a few proliferating fibroblasts in the anterior chamber and no exudate in samples from days 21 and 28. This demonstrates a considerable reduction in inflammation, which could be related to adequate concentrations of dexamethasone sodium phosphate release from the OCUGEL. Despite being long-standing, the drug-free specimens for day 28 confirmed a significant fibrinopurulent exudation in the anterior chamber. This is due to the lack of an anti-inflammatory agent to aid in the reduction of inflammation. This demonstrate the OCUGEL's efficiency in accelerating tissue remodelling performance when inflammation is triggered.

Keywords: ocular drug delivery, moxifloxacin HCL, dexamethasone sodium phosphate, biocompatible, TASS, wound healing.

Appendix E: Turnitin Report

A BIORESPONSIVE INJECTABLE OCULAR GEL SYSTEM FOR POST-SURGICAL CATARACT TREATMENT

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