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Development and characterization of a solid lipid nanoparticle-loaded thermosensitive gel for the delivery of timolol to the eye

Lynch, Courtney Rose

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DEVELOPMENT AND CHARACTERIZATION OF A SOLID LIPID NANOPARTICLE-LOADED THERMOSENSITIVE GEL FOR THE DELIVERY OF TIMOLOL TO THE EYE

COURTNEY ROSE LYNCH

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



Supervisors:

Professor Yahya E Choonara, Professor Pierre PD Kondiah, Professor Lisa C du Toit
Wits Advanced Drug Delivery Platform Research Unit, Department of Pharmacy and Pharmacology, School of Therapeutic Sciences, Faculty of Health Sciences, University of the Witwatersrand, South Africa

Dr Naseer Ally
Department of Ophthalmology, Faculty of Health Sciences, University of the Witwatersrand, South Africa

Johannesburg
2022

DECLARATION

I, Courtney Rose Lynch, declare that this thesis is my own work. It has been submitted for the degree of Master of Pharmacy in the Faculty of Health Sciences at the University of the Witwatersrand, Johannesburg, South Africa. It has not been submitted before any degree or examination at this or any other University.



……..

Signed on the …22….. day of ……March 2022……..

RESEARCH OUTPUTS

Publications

1. Lynch, C.R.; Kondiah, P.P.D.; Choonara, Y.E.; du Toit, L.C.; Ally, N.; Pillay, V. Advances in Biodegradable Nano-Sized Polymer-Based Ocular Drug Delivery. *Polymers* 2019, *11(8)*, 1371.
2. Lynch, C.R.; Kondiah, P.P.D.; Choonara, Y.E.; du Toit, L.C.; Ally, N.; Pillay, V. Hydrogel Biomaterials for Application in Ocular Drug Delivery. *Frontiers in Bioengineering and Biotechnology* 2020, *8,* 228.

International Research Presentations

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DEDICATION

This dissertation is dedicated to my grandfather, Peter Hickman.

Of all the giants' shoulders, yours are the tallest.

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I could not have accomplished anything in my life without my parents, Paul and Carole Lynch. I will forever appreciate your love and support in everything I do. To my siblings, Vicky and Richard, thank you for keeping my spirits up and supporting me always. To the rest of my family, my Nana, Uncles, Aunts and Grandparents, I am so lucky to have you all in my corner.

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ABSTRACT

The delivery of drugs to the eye is notoriously challenging. This is due to various physiological barriers which prevent the movement of foreign bodies and substances from getting into the eye, such as the cornea and conjunctiva. In addition to this, biomechanical processes such as the blinking reflex and rapid nasolacrimal drainage removes substances from the surfaces of the eye leading to a very short residency time. Currently, the first line treatment for most anterior segment conditions of the eye is the daily administration of drops. These solutions are known to have a low bioavailability, leading to frequent dosing schedules which are often not adhered to by patients correctly. This can be highly detrimental, especially in the case of glaucoma, a condition which is known to cause irreversible blindness due to increased intraocular pressure and subsequent optic nerve damage if not treated adequately from the time of diagnosis. In this study, an innovative formulation was developed composed of drug loaded solid lipid nanoparticles embedded within a thermosensitive gel was developed. The polymers employed for the gel were hyaluronic acid and methylcellulose, natural polymers which have been used previously in ophthalmic preparations and are known to be mucoadhesive and biocompatible. The choice of these polymers was aimed at increasing the residency time of the formulation at the surface of the eye to allow for better penetration through the layers protecting the eye. This is of particular importance as the current commercially available eye drops are known to be largely removed from the surface of the eye within 30 seconds of administration. The lipids selected were Compritol 888 ATO and oleic acid, and the SLNs were formulated using a nanoemulsion method. The formulated SLN-G system was characterized through various techniques including FTIR, TGA and DCS analysis. These characterizations showed a system in which the drug, timolol was effectively incorporated into the SLN with an encapsulation efficiency of 97.18% and a drug loading capacity of 56.12%. This encapsulation efficiency was further highlighted in the thermal analysis of the SLN-G, thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC), where the graphs of the drug loaded SLN-G did not show the characteristic peaks of timolol thereby confirming that it was effectively incorporated into the system. Results of the rheology studies revealed that the gel underwent the sol-gel transition at 33°C when not nano-enabled and at 28°C when embedded with the SLNs. These are appropriate temperatures for application at the surface of the eye. The average size of the SLNs was 54.75nm and they were spherical in morphology, as confirmed through SEM imaging. In addition to this, the *in vitro* drug release showed an extended drug release profile of approximately 24 hours in comparison to the commercially available product, which releases the drug instantaneously, and could potentially be administered once daily. The current first line glaucoma treatment, timolol eye drops are administered twice daily. By decreasing the frequency of the dosing schedule, patient compliance increases. The SLN-G and its components were also tested for biocompatibility using two different cell lines, Caco-2 and Human Retinal Pigment Epithelial (HRPE) cells. The cell viability was shown to be above 76% for the HRPE after 48 hours of exposure to the drug-loaded SLN-G and above 86% for the Caco-2 cell line, highlighting that the biocompatibility of the formulation. Through these results, it is proposed that an effective alternative to eye drops was developed.

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LIST OF ABBREVIATIONS

AMD – Age-Related Macular Degeneration

ATP – Adenosine Triphosphate

CMC – Carboxymethylcellulose Sodium

EDC – 1-ethyl-3-(3-dimethyl aminopropyl)

GTA – Glutaraldehyde

IOP – Intraocular Pressure

NZW – New Zealand White

PCL – Poly (ε-caprolactone)

PECs – Polyelectrolyte Complexes

PEG – Poly (ethylene glycol)

PLGA – Poly (lactic-co-glycolic acid)

PVA – Poly (vinyl alcohol)

RPCs – Retinal Progenitor Cells

SLN – Solid Lipid Nanocarrier

VEGF – Vascular Endothelial Growth Factor

CHAPTER 1

INTRODUCTION

1.1. Background to Ocular Drug Delivery

Glaucoma is the leading cause of irreversible blindness worldwide. The global prevalence of glaucoma in people over 40 years of age is projected to increase from 76 million in 2020 to 111.8 million in 2040 (Tham et al., 2014). Africa has the highest prevalence of glaucoma. It occurs at an earlier age, is more severe and often presents with one eye blind due to the disease at the time of diagnosis (Cook, et al., 2009). It is primarily an optic neuropathy that is associated with a raised intraocular pressure. Once optic nerve damage has occurred it is irreversible. The most common form of glaucoma is open-angle glaucoma. In this form, aqueous humor drainage through the trabecular meshwork is impaired even though the angle of the anterior chamber is not obstructed. Other forms of glaucoma include angle-closure glaucoma (where the angle through which the fluid drains becomes closed due to bulging of the iris), normal-tension glaucoma (where the optic nerve is damaged but with no apparent increase in intraocular pressure) and pigmentary glaucoma (where iris pigment granules build up in the trabecular meshwork and thereby prevent the drainage of fluid) (Rizzo et al., 2017).

Aqueous humor drains from the anterior chamber via 2 routes; through the trabecular meshwork, which is the conventional pathway (approximately 80% of outflow), or the uveoscleral outflow pathway, which drains through the ciliary body and into the supraciliary or suprachoroidal spaces (approximately 20% of outflow). The former pathway is pressure dependent whilst the latter is pressure independent (Goel et al., 2010).

The current treatment for glaucoma is aimed at lowering the intraocular pressure either through medicated eye drops, oral tablets or various surgical procedures. Eye drops, which are first line treatment, work in one of two ways; to increase the flow of aqueous humor through the uveoscleral outflow pathway or to decrease the rate at which the eye produces this fluid. The classes of active ingredients used in eye drop formulations include prostaglandin F-2α analogues (these increase fluid drainage, for example, bimatoprost), beta-blockers (these decrease fluid production, for example, timolol), diuretics or alpha agonists (these decrease fluid production as well as increase fluid drainage, for example, brimonidine) (Rizzo et al., 2017). The first line monotherapies are prostaglandin F-2α analogues. In addition, there are combination drops available (Conlon et al., 2017). Surgical methods used to treat glaucoma include laser trabeculoplasty, trabeculectomy, filtration surgery, the insertion of drainage tubes and minimally invasive glaucoma surgery (MIGS), which entails various surgical approaches (Mayo Clinic, 2019).

These current treatments face various challenges, including poor bioavailability and rapid clearance. Poor bioavailability of ocular drugs (administered as eye drops) is a result of the eye's advanced clearance mechanisms, including blinking, tear formation and the restoration of the tear film in a very short period. Tight cell junctions that are found in the eye also greatly restrict the movement of drugs into the eye. Furthermore, the prolonged use of eye drops has been shown to cause damage to the eye through inflammation and tear film instability, due to the preservative content (Thompson et al., 2018).

Ocular inserts have also been developed for the treatment of glaucoma. The most commonly used is Ocusert™, an insert which contains pilocarpine. The insert is designed to deliver the drug over a period of 7 days. However, these inserts have been reported to have complications including patients reporting that the device is uncomfortable and often falls out. Other issues include that the patient needs to be adequately educated in order to be able to use the insert correctly, which has been shown to be a challenge with elderly patients (Lavik et al., 2011). An implant has been formulated for the sustained release of bimatoprost; the implant is inserted intracamerally and releases the active ingredient over a 4-to-6-month period. It has undergone phase I/II clinical trials and has shown to have favourable efficacy and safety (Lewis et al., 2017). There are also inserts available for the treatment of various ocular conditions that are surgically inserted. Although these are able to administer an active ingredient for a longer period of time (examples, Ozurdex™ and Retisert™) there are drawbacks to these formulations, such as the invasive procedure needed to insert the product, the cost thereof, and the need for a surgical procedure to remove it (Lavik et al., 2011).

Although there are multiple eye drop formulations available on the commercial market, the bioavailability of the active ingredients and patient compliance remains a problem due to the fact that eye drops need to be instilled on a daily, sometimes twice daily, basis. In the case of glaucoma treatment, timolol eye drops are administered twice daily (Conlon et al., 2017). Topical administration of ocular drugs is the preferred method of treatment as it is easier for the patient to self-administer. However, there are several factors that need to be considered for preparations administered directly to the eye, such as sterility, viscosity, pH levels and particle size, to avoid causing discomfort in the eye which would lead to an increase in tear production. This further decreases the drug bioavailability, as it is washed away from the eye before it is able to be absorbed (Imperiale et al., 2018). Most of the patients who suffer from glaucoma are elderly patients and are visually impaired, both of which negatively impact patient adherence to treatment.

Many innovations have been made in recent years aimed at improving the delivery of drugs to the eye. These include the use of nanotechnology such as nanoparticles, nanomicelles, and nanofibers, as well as microneedles and liposomes (Gote et al., 2019). In particular, solid lipid nanoparticles (SLNs) are able to provide advantages to ocular drug delivery such as biocompatibility, low toxicity and sustained drug release profiles. However, there are currently no SLN formulations available on the commercial market, particularly for the treatment of glaucoma and other ocular conditions (Wang et al., 2018).

The proposed nanoparticle-laden ocular device in this study would alleviate the bioavailability and clearance shortfalls, as well as the frequent administration factor. This would aid in the patient adherence factor. The benefits of the proposed system over those of a surgical implant is that, in using a biodegradable polymer, it would not have to be surgically removed, and would still be able to deliver the active ingredients over a longer period of time.

1.2. Rationale and Motivation for the Study

This study focused on the development of a system which would improve the delivery of drugs to the eye, using hydrogel technology and nanotechnology. This system is aimed alleviating the challenges that are noted with the current treatment of glaucoma. The recommended dose of timolol (a beta-blocker) for the treatment of glaucoma is one drop into the eye twice a day. The eye drops have a concentration of 5mg/mL (Rossiter et al., SAMF, 2016).

Polymer-based nanosystems have been shown to increase the bioavailability of ocular drugs by retaining drug at the absorption site for a prolonged period compared to the formulations currently on the market which are washed away within a matter of minutes (Imperiale et al., 2018). This allows for less frequent dosing and an increase in patient compliance. Currently there are very few products available for sustained release of glaucoma therapy. A sustained release system, that can be administered into the anterior segment of the eye, will alleviate the need for twice daily administration of the drops.

It is proposed that a solid lipid nanoparticle (SLN) system, loaded within a polymeric, thermosensitive hydrogel, would be able to overcome the challenges of the current glaucoma treatment by increasing drug bioavailability and reduce the need for prolonged, daily eye drop administrations. SLNs are known to be biocompatible and provide sustained drug release profiles (Wang et al., 2018).

The preferred polymers identified for the hydrogel were hyaluronic acid (HA) and methylcellulose (MC). These are both natural polymers which are known to be biocompatible, biodegradable and non-immunogenic, making them an ideal choice in drug delivery systems (Anwunobi et al., 2011). HA is a polysaccharide which is composed of alternating units of D-glucuronic acid and N-acetyl-glucosamine. The high biocompatibility is due to the fact that HA is an endogenous substance which is found in a variety of ocular tissues including the cornea and the aqueous humor, rendering it particularly appealing for ocular drug delivery. HA is also known to adhere to the corneal layer through non-covalent bonds which aids in increasing the residency time of the formulation at the surface of the eye (Zhang et al., 2021).

MC is a cellulose derivative which has been used extensively ocular drug delivery. It is water soluble and forms a gel at a high enough temperature, between 50 and 72°C depending on the polymer concentration (Li, 2002). It is also known to be thermo reversible, meaning that it is able to return to a liquid state when cooled. Through an increased viscosity, formulations are not washed away from the surface of the eye as rapidly, thus increasing the residence time. The temperature at which MC undergoes a sol-gel transition can be altered through the addition of various polymers, both natural and synthetic, or salts (Bain et al., 2010).

Hydrogels have been largely investigated in various medical fields, including regenerative medicine and drug delivery. They are able to be fine-tuned to suit the environment into which it is being placed as well as its desired function. Adjustable characteristics including the biocompatibility, the *in situ* gelling properties, the swelling and the biodegradability (Pakulska et al., 2015). It is for this reason that more than one polymer was employed for this system.

SLNs, along with other colloidal systems, can enhance the penetration of drugs through the cornea of the eye. In addition to this, SLNs are able to enhance ocular tolerance and increase the corneal uptake. However, these are still a form of aqueous dispersion systems, which results in a rapid removal from the administration site. By incorporating the SLNs into a gel, the viscosity of the system is increased, leading to an improved corneal retention capability, as well as improved stability. *In situ* forming gels, which react to either a specific pH or temperature, offer a particular advantage in this regard as they can be administered as a liquid (in the form of an eye drop) and then undergo a sol-gel transition on the surface of the eye (Hao et al., 2014).

1.3. Aims and Objectives

The aim of the study was to design a nano-enabled drug delivery system for the delivery of an anti-glaucoma drug. The system would be comprised of solid lipid nanoparticles which are loaded within a polymeric thermosensitive gel.

The following objectives were defined for the study:

1. Development of solid lipid nanoparticles, loaded with the anti-glaucoma drug, to improve the penetration of the drug through the barriers of the ocular surface.
2. Preparation of a thermosensitive polymeric gel, into which the nanoparticles would be loaded, to overcome the rapid clearance of substances from the surface of the eye. The parameters of the gel are such that it is a liquid at room temperature and undergoes a sol-gel transition at body temperature.
3. Evaluation of the nano-enabled system via determination of the physicochemical properties. These properties would be investigated using x-ray powder diffraction (XRD), differential scanning calorimetry (DSC) and Fourier transform infrared (FTIR) spectra.
4. Evaluation of the physicomechanical properties of the nano-enabled system via texture and rheological analysis.
5. Optimization of the formulation in terms of *in vitro* drug release, viscosity and polymer degradation.
6. Investigation of cytotoxicity of the formulation through a 2,3-bis (2-methoxy-4-nitro-5-sulfophenyl)-5-[(phenylamino) carbonyl]-2H-tetrazo-lium hydroxide (XTT) assay.

1.4. Overview of Dissertation

This dissertation covers all aspects of the research including the pre-formulation design studies and *in vitro* critical evaluation.

Chapter 1 provides an inclusive overview of the rational and motivation for this research. A description of the system as well as the mechanism of action is given. The aims and objectives of the study are also explained, including the analytical sequence of the research.

Chapter 2 discusses in detail the current research which has been conducted on various polymers and nanotechnology systems in the field of ocular drug delivery. A detailed description of the challenges faced in ocular drug delivery is provided. A review of natural and synthetic polymers is given along with their inclusion in current research studies.

Chapter 3 discusses the employment of hydrogel technologies in ocular drug delivery. A description of the synthesis avenues of various hydrogel technologies is provided along with the inclusion of nanotechnology.

Chapter 4 discusses the development and formulation of the solid lipid nanoparticle-loaded gel (SLN-G) formulation. The formulation procedures as well as the various characterisations of the formulation, its components and the pristine ingredients are described. The cytotoxicity of the formulation is also investigated and described.

Chapter 5 discusses the conclusions of the study. In addition to this the future prospects of the formulation and further recommendations are described.

1.5. Concluding Remarks

This chapter provided an introduction and rationale for the research undertaken as well as the significance of this work in the field of ocular drug delivery and the treatment of glaucoma. This study would proposedly provide an insight into the development of a system which would overcome the challenges in delivering drugs to the eye and eliminating the need for frequent dosage administration, thus improving patient compliance.

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CHAPTER TWO

ADVANCES IN BIODEGRADABLE NANO-SIZED POLYMER-BASED OCULAR DRUG DELIVERY

2.1. Introduction

The eye is one of the most well-protected organs in the body. It is composed of many complex layers and structures, with a variety of defence mechanisms. These barriers, designed to protect the eye against foreign particles, molecules and infectious organisms, also greatly inhibit the movement of active ingredients into the eye. This presents a challenge when it comes to delivering drugs effectively to treat ocular conditions. Many of the formulations which are currently available on today's market experience low bioavailability and rapid clearance from the administration site, resulting in a frequent dosing schedule (Ameeduzzafar et al., 2018). The dosage frequency depends on the route of delivery; for example, liquid eye drop formulations are generally administered on a daily, if not 2–3 times a day, whereas intravitreal injections can be administered every 4 to 6 weeks (Brown et al., 2017; Gomes-Ballesteros et al., 2019).

The effective treatment of ocular conditions is of paramount importance. Many of these conditions, such as glaucoma (in which most therapies target the anterior segment) and age-related macular degeneration (AMD) which a posterior segment condition, negatively impact the patient's vision. If these conditions are not effectively treated and the vision impairment prevented, the damage or vision loss is irreversible (Gomes-Ballesteros et al., 2019; Brown et al., 2017). An impairment of vision can have devastating effects on a patient's quality of life. They are restricted or unable to perform everyday tasks, thereby resulting in limited physical activities (Vu et al., 2005).

When considering the challenges that are faced in delivering drugs to the eye, many innovations are being made, employing both polymers and nanosystems in order to optimize this route of drug delivery. Polymers, specifically biodegradable polymers, offer a number of benefits when it comes to enhancing therapeutic ophthalmic formulations. The primary benefit is their mucoadhesive property, especially in the region of the cornea and conjunctiva. This allows for a formulation to have an increased residence time on the corneal epithelial surface which allows for improved drug penetration (Ludwig, 2005).

Nanoscaled drug delivery systems have been widely investigated in order to optimize ophthalmic therapeutic preparations. They provide a number of benefits, ranging from sustained drug release profiles to improved permeation through ocular barriers. Not only are they able to improve

formulations used to treat the anterior segment of the eye, they are also able to improve drug delivery to the posterior segment of the eye, a region that is notoriously difficult to treat (Xu et al., 2013).

This review aims to provide an overview on recent advances in ocular drug delivery and limitations of the conventional delivery systems on the market. In doing so, the importance of biodegradable polymers and nanotechnology in this field of pharmaceutical development will be highlighted by illustrating current challenges faced in ocular drug delivery and how they can be exponentially modified for greater ocular bioavailability.

2.2. Ocular Physiological Defence Mechanisms for Drug Delivery

The first defence mechanism involves the structures anterior to the eye, such as the eyelid and lashes. These structures, along with the body's blinking reflex, are able to defend the eye against intrusion of particles which could cause mechanical damage to the eye if they were to come into contact with it. This blinking reflex plays a role in the challenges faced by current ocular drug delivery systems by rapidly removing the formulation from the surface of the eye and thereby not allowing the drug adequate time to penetrate through the barriers, which will be described in detail below (Sack et al., 2001).

The eye is divided into two segments; the anterior segment (which encompasses the tear film, cornea, conjunctiva, iris, ciliary body, aqueous body, lens and anterior sclera) and the posterior segment (which encompasses the posterior sclera, choroid, Bruch's membrane, retina and vitreous humor). There are a myriad of conditions which impact the anterior or posterior segments respectively and each of these requires treatment in one way or another. Although drug delivery to the anterior segment of the eye is not without its challenges, conditions which affect this segment of the eye are easier to treat because the active ingredients are able to be applied topically, which is easier and less invasive for the patient (Barar et al., 2016).

Drugs that are administered to the surface of the eye are removed via the nasolacrimal drainage system and are absorbed by the nasal mucosa. This not only prevents the active ingredient from entering the aqueous humor but can also result in unwanted side effects as it enters systemic circulation (Gupta et al., 2010).

Ocular active ingredients are also removed from the eye via efflux transporters. These transporters are ATP-binding proteins (adenosine triphosphate binding proteins) that are responsible for

protecting cells by removing various substances such as drugs, metabolic compounds and lipids. The efflux transporters which are most prominently expressed in ocular epithelium are P-glycoprotein and multidrug resistant protein. Multiple studies have assessed that administering active ingredients that inhibit these transporters along with ocular active ingredients may help to improve the effectiveness of ocular drug delivery systems (Rawas-Qalaji et al., 2012).

Ocular immune privilege was first described by Sir Peter Medawar when he observed that grafts, composed of tissues foreign to the eye, were not rejected when they were placed into the anterior chamber. It was initially thought that the immune privilege in the eye is due to the fact that there is no direct lymphatic drainage from the eye as well as the presence of the blood-ocular barrier, which prevents the entry of substance into the aqueous humor from the systemic circulation (Taylor, 2009). However, it has been reported that this process is much more complex, as other factors such as the presence of immunodulatory factors (namely AqH) and antigen-presenting cells play a significant role. The quality of immune privilege allows for the eye to have an immune response to a foreign pathogen, without causing damage to local tissues which is usually an effect of an immune response and the inflammatory process that follows. This allows for foreign substances such as ophthalmic formulations to be administered to the eye and enter both the anterior segment and the posterior segment without loss of the specialized tissue cells responsible for the sense of sight (Streilein, 2003).

This review will highlight how each of the main ophthalmic barriers to ocular drug delivery can be overcome with the use of polymeric nanosystems. Figure 1 illustrates structures within the eye that hinder the absorption of administered drug. Many of these act as a barrier to the delivery of drug to the eye, as discussed below. The two blood-ocular barriers are also illustrated (Huang et al., 2019).

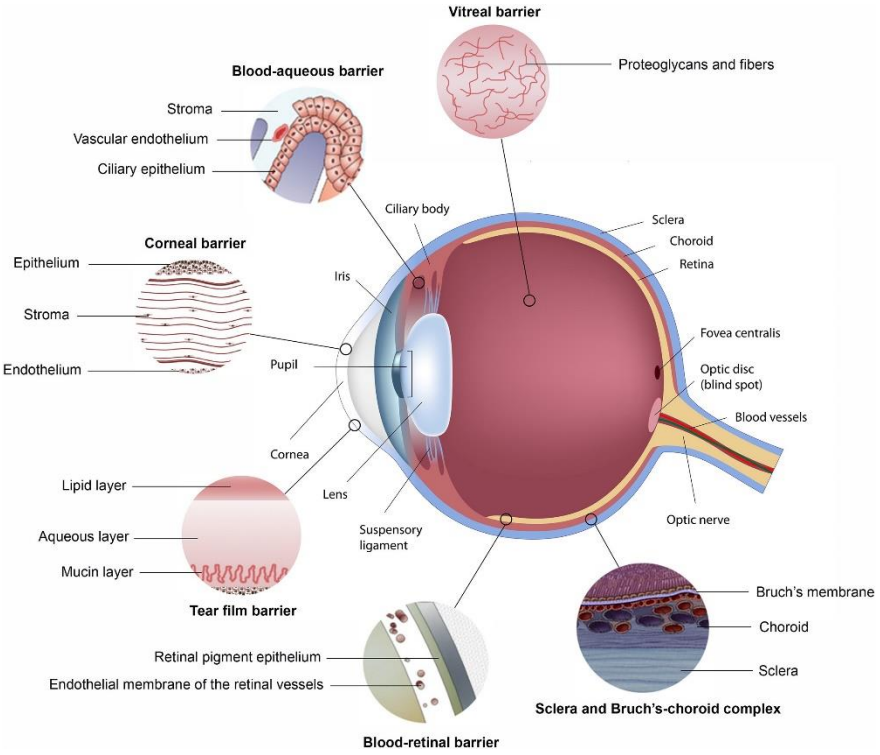


Figure 2.1. Ocular physiological defences that provide barriers for the delivery of drugs to segments of the eye, with each barrier highlighted, preventing drugs from penetrating across each membrane (Huang et al., 2019).

2.2.1. Cornea

The cornea forms one of the major barriers for the transport of substances into the eye. This barrier, which is made up of multiple layers of tissue (the corneal epithelium, Bowman's layer, the stroma, Dua's layer, Descemet's membrane and the endothelium), is kept moist by tears. Lacrimation and tear turnover are also responsible for flushing away any matter which is foreign to the eye, including a large quantity of an administered formulation (Ramsey et al, 2018). The tight cell junctions within the cornea prevent molecules from moving into the eye (Akpek et al., 2003). Due to the lipophilic properties of the corneal epithelium, hydrophilic molecules are largely unable to permeate through it. The washing away of formulations which are applied to the eye is largely due to the rapid turnover rate of lacrimal fluid. This results in the active ingredient being removed from the surface of the eye, where it would permeate through the above-described layers, to the nasolacrimal duct in only a matter of minutes (Tsai et al., 2018).

Polymeric nanosystems are able to adhere to the mucosa of the eye, leading to an increased residence time at the cornea. This, along with the small particle size of nanosystems, allows for increased drug permeation across the cornea (Mandal et al., 2017).

2.2.2. Conjunctiva

The conjunctiva is comprised of a thin membrane which is responsible for the production of tears as well as the maintenance of the tear film. There is also a rich supply of vasculature, such as capillaries and lymphatic drainage within the conjunctiva, resulting in the removal of active ingredient molecules (Kuno et al., 2011). These blood vessels do not contain tight cell junctions, so drugs that are administered locally are able to enter systemic circulation. This entry of active ingredients into the blood stream via the conjunctiva can lead to systemic side effects when the formulation is administered to the eye (Ameeduzzafar, 2014; Huang et al., 2019).

Polymeric nanosystems are able to provide mucoadhesive properties which prevents drugs from being removed by these mechanisms within the conjunctiva.

2.2.3. Sclera

The sclera, a collection of collagen fibres and proteoglycans, is both part of the anterior and the posterior segment of the eye. However, it is thicker towards the posterior segment, and responsible providing mechanical support thus for giving the eye its shape (Ameeduzzafar, 2014). The permeation of drug molecules through the sclera is determined by the molecules' hydrophobicity (the more lipophilic the molecule, the less it diffuses through) and charge (positively charged molecules bind to the proteoglycan matrix within the sclera and are unable to diffuse through) (Kuno et al., 2011). The sclera has been considered a viable option for the delivery of active ingredients to the posterior segment of the eye due to the fact that it has a large surface area, is accessible and is relatively permeable (Geroski et al., 2000). Recently, a study was conducted to develop and investigate polymeric particles that are able to be guided by magnetic forces across the sclera. This resultantly prevented the drug from being removed via bulk fluid flow from the posterior segment of the eye (Mousavikhamene et al., 2017).

2.2.4. Blood-Ocular Barriers

There are two blood-ocular barriers which largely prevent the use of systemically administered active ingredients to treat ocular conditions, namely the blood-aqueous barrier and the blood-retinal barrier, as detailed in Figure 1. Although some active ingredients are able to pass through these barriers (such

as those with high lipophilicity or with the aid of an active transport system), such a small volume of blood flows through the vasculature within the eye that a high dose would be needed, leading to systemic side effects (Eljarrat-Binstock et al., 2010; Gaudana et al., 2010).

2.2.5. Posterior Segment

Treatment to the posterior segment of the eye often includes invasive procedures such as surgery, implantation of a drug loaded device or injectable therapy (either intravitreally or subconjunctivally), due to the fact that active ingredients that are topically applied are not able to reach the vitreous humor in effective concentrations (Geroski et al., 2000).

The retina forms part of the posterior segment of the eye. It is responsible for our ability to see. Diseases affecting the retina include AMD and diabetic retinopathy (Kaji et al., 2018). It forms part of the blood-retinal barrier. Delivery of drugs to the retina is challenging and could benefit greatly from the development of polymeric nano-drug delivery systems (Bisht et al., 2018).

Each of these barriers plays a role in limiting the entry of active ingredients into the eye, leading to the low bioavailability of many ophthalmic formulations.

2.3. Current Limitations of Conventional Delivery Systems Employed for Ocular Therapeutics

Ophthalmic conditions and diseases are treated using a variety of applicable administered methods. Each of the methods currently on the market has its own limitations, advantages and disadvantages both in terms of the delivery of the drug, and also from a patient's compliance perspective. A summary of these is provided below in Table 1.

2.3.1. Eye Drops (Solutions and Suspensions)

The current first line treatment for many ocular conditions, particularly those affecting the anterior segment of the eye, is eye drops. However, traditionally, these formulations have a low bioavailability and a rapid clearance from the administration site due to the defence mechanisms, as highlighted above. Researchers have tried to enhance the bioavailability of topical eye drop preparations in a number of ways, such as the addition of penetration enhancers and viscosity modifiers (Gaudana et al., 2010).

Due the direct administration of drops to the eye, it is important to consider the limit to dosage size that the cul-de-sac of the eye can withstand; usually between 7 and 10 µL. It has been shown that

approximately 10% of an administered dose of eye drops (approximately 50 µL) will penetrate into the eye and reach the target site at an effective concentration. This often leads to a frequent dosing schedule (Eljarrat-Binstock et al., 2010; Mohammed et al., 2019). The frequency at which some eye drops have to be administered have been shown to cause damage to the precorneal film of the eye. Benzalkonium Chloride (BAK), a quarternary ammonium, is the most commonly used preservative in eye drops. It acts as a detergent, and although highly effective against bacteria, it damages the lipid layer of the tear film. BAK has also been implicated in decreasing the number of goblet cells, leading to reduced mucin production and tear film instability. It also has a direct, dose dependent toxic effect on the corneal epithelium and has been shown to reduce the number of microvilli on the epithelial surface. All of these changes cause causing evaporative dry eye (Asiedu et al., 2019).

Although there are challenges when it comes to topical ocular drug delivery, which is a drawback to this method of treatment, it must be noted why it is one of the primarily used ocular treatment regimens. Topical ocular preparations are able to be self-administered and are non-invasive for the patient (Gaudana et al., 2010). It is thus understandable why a patient would opt for topical treatment rather than another option which is more invasive.

However, patient compliance to eye drops is low. One must consider the inconvenience of daily eye drop administrations in patients with chronic ocular conditions, such as glaucoma. Research has shown that a large number of patients, who are supposed to be on treatment indefinitely, stop receiving their medication after a period of time and discontinue treatment (Subrizi et al., 2019; Mohammed et al., 2019). A study has shown that the adherence rate of patients who are using eye drops for the treatment of glaucoma ranges between 30% and 80%. The reasons for the low level of adherence, according to the literature, range from a lack of understanding for the need to administer treatment regularly, to the cost of medication. Other reasons include patients simply forgetting to administer it (Nordstrom et al., 2005).

Eye drops allow the patient to administer their medication themselves at home. However, there are limitations to this. As previously described, due to the low bioavailability and rapid clearance of these formulations, patients often have frequent dosage regimens. This then further decreases patient adherence. Patients also need to be educated on the correct way to use and store their medications in order to prevent the spread of infection from either from one eye to the other, or from one patient to another (Newman-Casey et al., 2015).

The use of both polymers and nanosystem can be employed in optimizing eye drops to have an increased residency time, improved permeability into the target site and better bioavailability. This will lead to less frequent dosing schedules, for example, changing a dosing regimen from two or three times a day administration to daily or weekly administration, will in turn help to improve the rate of patient adherence.

2.3.2. Ointments

Ointments for the delivery of ocular active ingredients have been developed extensively to date. Due to the viscous nature of the formulation, they are not washed away from the eye as rapidly as liquid formulations, resulting in a higher bioavailability. However, this viscosity also leads to temporary blurred vision and inaccurate dosing (Eljarrat-Binstock et al., 2010). For this reason, white petrolatum is often used as an ointment base. It has a suitable melting point which will result in a decreased viscosity once it has been administered to the eye (Bao et al., 2017).

Although ointments are advantageous in terms of bioavailability, they are not investigated as extensively as other ophthalmic formulations. This could be due to the fact that they have a number of formulation challenges such as poor content uniformity and reproducibility (Bao et al., 2017).

2.3.3. Intravitreal Injections

Injections directly into the vitreous humor are often used for the delivery of drugs to the posterior section of the eye. For example, anti-Vascular Endothelial Growth Factor (VEGF) drugs are primarily administered intravitreally for the treatment of AMD and diabetic macular oedema. However, besides the fact that the procedure is invasive and unpleasant for the patient, these injections carry a number of risks. Major risks include possible endophthalmitis or retinal detachment, among others. These injections also have to be administered fairly frequently (for example, in the case of AMD treatment, every 4 to 6 weeks), resulting in poor patient compliance. Thus, the sustained drug release benefits that are found in nanostructures, further employing polymeric ocular drug delivery, could be utilized to extend the frequency at which these injections are to be administered (Bode et al., 2018; Del Amo et al., 2017).

2.3.4. Intraocular Implants

Another form of ocular drug delivery that has been developed are intraocular implants. Intraocular implants are surgically inserted into the eye where they release the drug over an extended period of time. Initially, these implants were not biodegradable and had to be surgically removed, which

portrayed a number of risks associated with this application. However, the investigation of biodegradable polymers in the development of ocular implants transformed the application for surgical removal (Bode et al., 2018). Implants are also able to be engineered as stimuli-responsive delivery systems. Currently, implants that are on the market, while being able to deliver drugs over a long period of time, are not able to change the rate at which they release drug. The investigations into stimuli-responsive implants are largely due to the developments which have been made employing stimuli-responsive polymers (Yasin et al., 2014).

2.3.5. Contact Lenses

For more than a decade, contact lenses have been investigated for the use in ocular drug delivery. In contrast to eye drops, contact lenses are able to offer an increased residence time, allowing for improved drug delivery. However, it has been shown that contact lenses release the majority of the drug over the first few hours (Torres-Luna et al., 2019).

This drug delivery system works by diffusing the active ingredient out of the lens matrix, where it is able to come into contact with the surface of the eye, allowing for permeation (Paradizo et al., 2016; Zinming et al., 2008).

However, the use of contact lenses as a drug delivery carrier system does pose risks. Patients must be informed on the correct, hygienic procedure when it comes to placing the lenses on the eye as well as taking them out in order to prevent the risk of infection. Sleeping with contact lenses in has been documented to lead to keratitis (Paradiso et al., 2016). Thus, by developing nanoparticulate drug-loaded polymeric biodegradable lenses, the above limitations will be overcome, achieving site specific targeting over a controlled period of time.

2.3.6. Emulsions

There are a number of ocular emulsions currently on the market. The most common form of emulsion currently used for drug delivery is an oil in water emulsion, due to the fact that it is better tolerated by the eye than a water in oil emulsion. Emulsions have been shown to increase the bioavailability of an active ingredient by improving the residence time and permeation through the cornea. A study using azithromycin as an active ingredient also showed a sustained drug release profile, in comparison to an azithromycin suspension (Patel et al., 2013).

However, ocular emulsions are unstable and susceptible to flocculation. They are also destabilized by tear fluid (Tamilvanan et al., 2004).

By employing nanoemulsion techniques, this problem can be circumvented, enabling uniform dispersity within both phases of the emulsion formulation, delivering the drug in controlled pharmacokinetic profiles.

Table 2.1. Advantages and disadvantages experienced by current conventional ocular drug delivery systems.

Dosage Form	Advantages	Disadvantages	References
Eye Drops (Solutions and Suspensions)	Ease of administration. Little discomfort to the patient. Non-invasive.	Low bioavailability. Limit to dosage size. Frequent administration. Low patient adherence.	(Gaudana et al., 2010; Mohammed et al., 2013; Eljarrat-Binstock et al., 2010; Gomez-Ballestros et al., 2019; Nordstrom et al., 2005)
Ointments	Ease of administration. Decreased clearance rate following administration. High bioavailability than liquid formulations.	Blurred vision. Inaccurate dosing. Challenges in formulating.	(Eljarrat-Binstock et al., 2010; Bao et al., 2017)
Intravitreal Injections	Drug administered directly to posterior segment.	Invasive. Multiple risks with frequent administrations. Frequent administration.	(Bode et al., 2018; Del Amo et al., 2017)
Intraocular Implants	Extended drug release. If biodegradable polymers used, no need for removal.	Surgical implantation and removal if not biocompatible.	(Bode et al., 2018; Yasin et al., 2014)

	Able to be developed as stimuli-responsive.	Risks associated with insertion. Insertion uncomfortable for patients.	
Contact Lenses	Increased residence time compared to other formulations. Keeps drug in contact with the surface of the eye for improved permeation.	Rapid release of drug within the first few hours. Cannot be used continuously. Risk of infection.	(Torres-Luna et al., 2019; Paradiso et al., 2016)
Emulsions	Improved bioavailability over other formulations. Improved residence time. Sustained drug release profiles.	Susceptible to flocculation and instability. Destabilized by tear fluid.	(Patel et al., 2013; Tamilvanan et al., 2004)

2.4. Biodegradable Polymers in Ocular Drug Delivery

Polymers, both synthetic and naturally occurring, have been used for a number of applications in the medical field, including improved drug delivery, 3-D printing and tissue engineering, attributed to their biocompatible nature. Polymers are being considered more effective for pharmaceutical formulations, as they are able to improve the dosing of a particular active ingredient. They are also able to lower the side effects experienced by a patient by allowing implantation into the diseased tissue, thus providing increased drug loading at the site while reducing the systemic concentration (Peterson, 2004).

In ocular drug delivery, polymers are widely accepted as being able to optimize a formulation through their mucoadhesive properties. This prolongs the time that the formulation is in contact with the cornea and conjunctival epithelium, helping to alleviate the challenge of rapid clearance from the eye which is often experienced by topical ocular formulations (Di Colo et al., 2009).

Certain polymers have been shown to have viscosity modifying properties, in addition to their mucoadhesive properties. When a polymer is being included in a formulation for its ability to increase viscosity, it is important to consider that if a solution becomes too viscous it will cause irritation to the

eye. This prompts a defensive reaction in the eye, further increasing the production of tears and subsequent clearance of the formulation. It has been suggested that polymers should be added to a formulation in order to increase mucoadhesive without drastically altering the viscosity (Di Colo et al., 2009).

A further benefit to the use of certain polymers is their ability to biodegrade. This means that, once they have been administered, they are broken down by the body in non-toxic components. As the polymer is broken down the drug is released, resulting in sustained drug release profiles. The ability to biodegrade allows for a drug delivery system to be administered without the need for manual removal. This is of particular interest in the development of ocular implants, as it removes the need for surgical removal of the implant (Kimura et al., 2001).

Certain polymers have been shown to be stimuli-responsive, allowing them to react or release an active ingredient upon a change in conditions, such as changes in temperature, pH or pressure. These are also known as “smart” polymers and have been used *in situ* as gelling systems; formulations that undergo a change in viscosity as a response to a change in physiological conditions. This process allows the formulation to remain at the site of administration longer, thus allowing more time for the active ingredient to permeate through the cornea (Almeida et al., 2014; Geethalakshma et al., 2013).

Bioresponsive polymers themselves are able to undergo a number of changes in response to a stimuli. These include changes in permeability, shape changes or phase separation, among others. This allows for the drug to be released from the formulation when it is needed (Bawa et al., 2009). The benefits of bioresponsive polymers can be seen in the developments that are being made in the field of ocular drug delivery. For example, implants developed to respond to inflammatory responses within the eye, have been investigated by many researchers to date (Du Toit et al., 2014).

Although many new formulations are being developed using nanotechnology, polymers are also being used to enhance or optimize older formulations. It has been shown that by adding polymers with mucoadhesive or viscosity modifying properties to an eye drop formulation, it is retained at the site and thus increases the bioavailability of the drug (Almeida et al., 2014; Irimia et al., 2018). Figure 2 depicts how a polymer, specifically chitosan, interacts with the mucin layers in the eye, reacting to mucoadhesive properties *in situ* (Irimia et al., 2018).

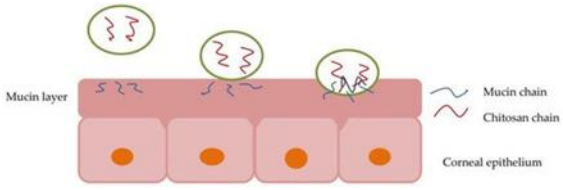


Figure 2.2. Illustration of **c**hitosan interaction with the mucin layer of corneal epithelium, allowing particle permeation via mucoadhesion (Irimia et al., 2018).

A combination of polymers is often used in a single formulation. This is done in order to overcome one or more disadvantages that a certain polymer may have. For example, when used in a hydrogel, chitosan has been shown to have poor mechanical strength and low elasticity. However, when another polymer is added, such as poly (vinyl alcohol) (PVA), a more suitable hydrogel is formulated (Irimia et al., 2018).

Many of the polymers discussed below are able to form polyelectrolyte complexes (PECs). These are formed when a positively charged polymer (such as chitosan) interacts with a negatively charged polymer (such as alginate) to form a cross-linked system. These complexes are then able to be utilized in the formation of nanoparticles and employed in drug delivery (Quinones et al., 2018).

The figure below (Figure 3) illustrates the chemical structure of each of the polymers (both natural and synthetic), as discussed in more detail below.

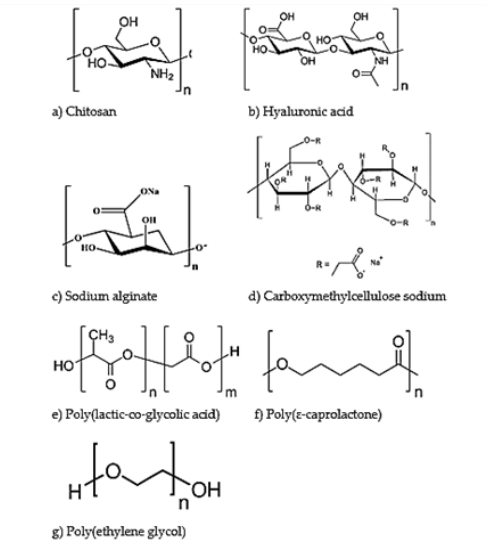


Figure 2.3. Chemical structures of biodegradable polymers employed for ocular drug delivery. The natural polymers discussed are chitosan (**a**), hyaluronic acid (**b**), sodium alginate (**c**) and CMC sodium (**d**). The synthetic polymers discussed are poly (lactic-co-glycolic acid) (**e**), poly (ε-caprolactone) (**f**) and poly (ethylene glycol) (**g**).

2.4.1. Natural Polymers

2.4.1.1. Chitosan

Chitosan, which is derived from chitin, is often used as a polymer in ophthalmic preparations (chemical structure seen in Figure 3a). It is a cationic polysaccharide, allowing it to react with the negative charges found within the mucus and conjunctiva of the eye. Although chitosan has a number of beneficial properties, it is only soluble in acidic mediums, which would cause irritation if placed into the eye without being completely neutralized. This has led to the development a number of derivatives, such as galactosylated chitosan and thiolated chitosan, which have more favorable solubility profiles for ocular drug delivery (Zhao et al., 2017; Zhu et al., 2012).

Chitosan has a number of favorable characteristics; it is biocompatible, mucoadhesive, non-cytotoxic, as well as biodegradable (Bhatta et al., 2012; Cheung et al., 2015). It has been shown to increase the retention time of the formulation once it has been administered, as well as improve the penetration of the drug through the cornea by opening the tight cells junctions that are present within the epithelial tissue, as seen in Figure 2 (Irimia et al., 2018). The effect of chitosan on tight cell junctions and subsequent increase in permeability was shown using Caco-2 cells (Yeh et al., 2011).

Chitosan has also been used in a number of developing ocular formulations because it has inherent antimicrobial and wound healing properties (Silva et al., 2017). A modified derivative of chitosan, chitosan-N-acetylcysteine has been shown to increase the rate of corneal healing in New Zealand White (NZW) rabbits when administered as eye drops twice daily (Fischak et al., 2017). The antimicrobial properties are derived from chitosan's positive charge, allowing it to inhibit microbial growth through binding to the membrane wall of the microbe thus causing permeability changes (Zhao et al., 2018).

This polymer, and its derivatives, is being developed into a number of nanotechnology formulations to treat a range of ocular conditions, such as nanoparticulate systems to treat glaucoma, conjunctivitis as well as multiple immune related ocular degenerative conditions (Silva et al., 2017; Fischak et al., 2017; Zhao et al., 2018).

2.4.1.2. Hyaluronic Acid

Hyaluronic acid is a natural polymer found in numerous sites within the human body including the eye (chemical structure shown in Figure 3b) (Liu et al., 2019). It has been investigated for use in ocular drug delivery systems because it is biocompatible, biodegradable and mucoadhesive. It is often used

in combination with chitosan or other polymers (Silva et al., 2017). A report by de la Fuente et al. highlights how hyaluronic acid can be used in conjunction with chitosan in a nanoparticulate drug delivery system for the administration of ocular gene therapy to both corneal and conjunctival cells (De la Fuente et al., 2008).

Hyaluronic acid is a negatively charged polysaccharide. Like chitosan, hyaluronic acid is often modified and its derivatives used not only in drug delivery, but also other aspects of the medical field, such as tissue engineering (Bongiovi, 2017). It has been used to prolong the retention time of many ocular active ingredients such as timolol, pilocarpine and gentamycin through its mucoadhesive and viscosity modifying properties. Hyaluronic acid also acts as an active ingredient in certain formulations for the treatment of dry eye disease or artificial tears (Salzillo et al., 2016; Graca et al., 2018).

Hyaluronic acid is able to be functionalized in order to modify its properties in the formulation. This is possible with compounds such as tyramine (Egbu et al., 2018), adipic dihydrazide and methacrylic anhydride (Widjaja et al., 2013). These researchers then formulated the functionalized hyaluronic acids into a respective hydrogel. These hydrogels could then be loaded either with a drug on its own, or with liposomes containing the drug (known as hyaluronic acid-based nanocomposite hydrogels). Through these drug delivery systems, researchers were able to obtain sustained drug release profiles. Thus, along with hyaluronic acid's biocompatible and biodegradable properties, studies have demonstrated optimization of drug delivery to the eye achieving significantly improved pharmacokinetic properties (Egbu et al., 2018; Widjaja et al., 2013).

Recently, hyaluronic acid has been used to formulate micelles, which were shown to be able increase the permeation of lipophilic ocular actives such as dexamethasone as well as reduce the rapid clearance rate from the eye (Bongiovi, 2017).

2.4.1.3. Sodium Alginate

Alginates are naturally occurring anionic polysaccharides that are largely used for their gelling abilities, allowing for viscosity modification when used in ocular formulations. They are derived from the cell walls of brown algae, as well as bacterial strains (Szekalska et al., 2016).

Sodium alginate is often used in ocular preparations due to the fact that is biocompatible, biodegradable, and allows for enhanced permeation (chemical structure shown in Figure 3c). However, it is very susceptible to enzymatic degradation. It is possible to modify sodium alginate using

other polymers (such as poly (lactic-co-glycolic acid)) in order for it to function as desired (Lin et al., 2019).

Sodium alginate has been employed largely due to the fact that, in addition to its ability to alter the viscosity of a formulation, it is mucoadhesive and allows for sustained drug release. This helps to overcome some of the challenges experienced by topical ocular formulations (Shelley et al., 2018).

Costa et al. reported the formulation of chitosan-coated alginate nanoparticles as a possible ocular delivery system for daptomycin. The researchers reported that the addition of sodium alginate to a nanoparticle formulation allows for a better sustained drug release profile than nanoparticles that only contain chitosan (Costa et al., 2015).

2.4.1.4. Carboxymethylcellulose Sodium

Carboxymethylcellulose (CMC) sodium is a natural polymer that has been shown to possess thermoresponsive properties (chemical structure shown in Figure 3d). It is currently used in formulations to treat dry eyes (Al-Kinani et al., 2018; Graca et al., 2018). Cellulose-derivative polymers are often used in ophthalmic preparations, due to their viscosity modifying abilities. Methylcellulose in particular has been shown to have ocular wound-healing properties, as well as the ability to act as a tear substitute (Ludwig, 2005).

Jain et al. reported the formulation of a polymeric membrane composed of sodium CMC and PVA, a water-soluble polymer that has been previously used in ophthalmic formulations. Sodium CMC allowed for the formulation to have mucoadhesive and biodegradable properties, while the addition of PVA improved the rigidity of the membrane. These inserts could have applications in the sustained delivery of drugs to the eye (Jain et al., 2010).

2.4.2. Synthetic Polymers

2.4.2.1. Poly (lactic-co-glycolic acid)

Poly (lactic-co-glycolic acid) (PLGA) is a synthetic, biodegradable polymer (chemical structure shown in Figure 3e). It has been largely investigated for drug delivery, tissue engineering and biodegradable sutures. This is mainly due to its biocompatibility and sustained release profiles as well as its ability to degrade in an aqueous medium. It has been formulated into nanostructures using a number of methods, including solvent evaporation and nanoprecipitation. PLGA can also be modified with the use of copolymers, such as poly (ethylene glycol), in order to enhance its characteristics. This has been

shown in a study conducted by Vasconcelos et al., employing ocular drug delivery for sustained release kinetics of PLGA-PEG nanoparticles. These nanoparticles were conjugated with a peptide and provided a promising ocular drug delivery application, due to their low toxicity, sustained drug release and high entrapment efficiency profiles (Mir et al., 2017; Vasconcelos et al., 2015).

PLGA has been documented to also be used in the development of a biodegradable implant which is capable of delivering dexamethasone to the posterior segment of the eye. Studies have also investigated using PLGA nanoparticles, capable of delivering drug to the posterior segment of the eye after topical administration (Tahara et al., 2017). Thus, the use of PLGA, conjugated to other polymers, has to date significant applications in ocular drug delivery, ranging from simply improving drug permeation to increasing the residence time of nanoparticulate systems.

2.4.2.2. Poly (ε-caprolactone)

Poly (ε-caprolactone) (PCL) is a synthetic polymer that has been commonly used in drug delivery due to its high biocompatibility and biodegradability (chemical structure shown in Figure 3f). It has been used in developments in ocular drug delivery, due to its ability to prolong drug release profiles (Lee et al., 2017). PCL is able to be formulated into thin films as well as polymer solutions with low toxicity and slower degradation than that of PLGA (Cao et al., 2019).

It has been used in drug delivery systems such as implants that are able to release the active ingredient over a period of four months or more. PCL was formulated into a micro episcleral film, which was able to deliver triamcinolone acetonide over a four-month period, preventing the development of proliferative vitreoretinopathy after intraocular surgery or trauma (Sun et al., 2016).

Investigations into PCL use have shown that when it is used in conjunction with nanoscale drug delivery, such as nanoparticles, it has demonstrated greater concentration of both indomethacin and cartelol in the aqueous humor (Sinha et al., 2004). Da Silva et al. have also reported the development of dexamethasone acetate loaded PCL nanofibers, which could be administered intravitreally post-surgery (Da Silva et al., 2015).

2.4.2.3. Poly (ethylene glycol)

Poly (ethylene glycol) (PEG) is a non-ionic, synthetic polymer with hydrophilic properties (chemical structure shown in Figure 3g). It has been used in the development of ocular drug delivery formulations, both on its own and in conjunction with other polymers such as PLGA. The addition of PEG to a nanoparticle formulation has been shown to increase their mucoadhesive properties

(Vasconcelos et al., 2015). An example of PEG being used in combination with other polymers is a report by Shi et al., wherein a nanosuspension composed of methoxy PEG-PCL and chitosan was formulated containing diclofenac. The study showed improved pre-corneal retention as well as penetration of the formulation across the epithelial membrane, resulting in a higher concentration of diclofenac entering the aqueous humor than that seen with commercial eye drops (Shi et al., 2015).
PEG has been used to enhance the performance of nanoparticles by increasing their permeation abilities. It also helps to prevent aggregation of nanoparticles, thereby improving their stability (Balguri et al., 2017).

PEG was developed into a lipo-polymeric nanoparticulate system for the delivery of ketoconazole. This allowed for increased permeation of ketoconazole through the epithelia of the cornea resulting in an improved bioavailability (Kakkar et al., 2015). Another study reported lower particle elimination through tear clearance, employing mucus penetrating particles of PEG-PLGA. A low PEG density was found to significantly bind to mucin, compared to the high-density PEG, which demonstrated much lower binding affinity (Figure 4a). Drug KPI-121, formulation of 0.4% loteprednol etabonate (LE), coated with Pluronic (F127), significantly improved pharmacokinetic properties in a NZW rabbit model, in comparison to Lotemax1 0.5%, a commercially available suspension of LE (Figure 4b). It was further noted that KPI-121 (0.4%) of a single topical administration, increased Cmax of the drug by 3-fold, quantified in aqueous humor, conjunctiva and the cornea, when evaluated against the commercial product (Figure 4c). Bioavailability analysis further proved double potential of the polymeric delivery system, against the commercially available LE product, in respective ocular tissues of the cornea, conjunctiva and in aqueous humor [76]. Thus, the application of employing mucus penetrating polymeric platforms have great potential in increasing ocular bioavailability in the aqueous humor, cornea and the conjunctiva. Polymeric delivery systems, such as PEG, will consequently increase patient compliance by decreased dosing frequencies, portraying significant retention time in ocular tissue, especially the conjunctiva, which has more than 5-times greater surface area than the cornea, allowing mucin to retain greater concentration of the administered drug (Meng et al., 2019).

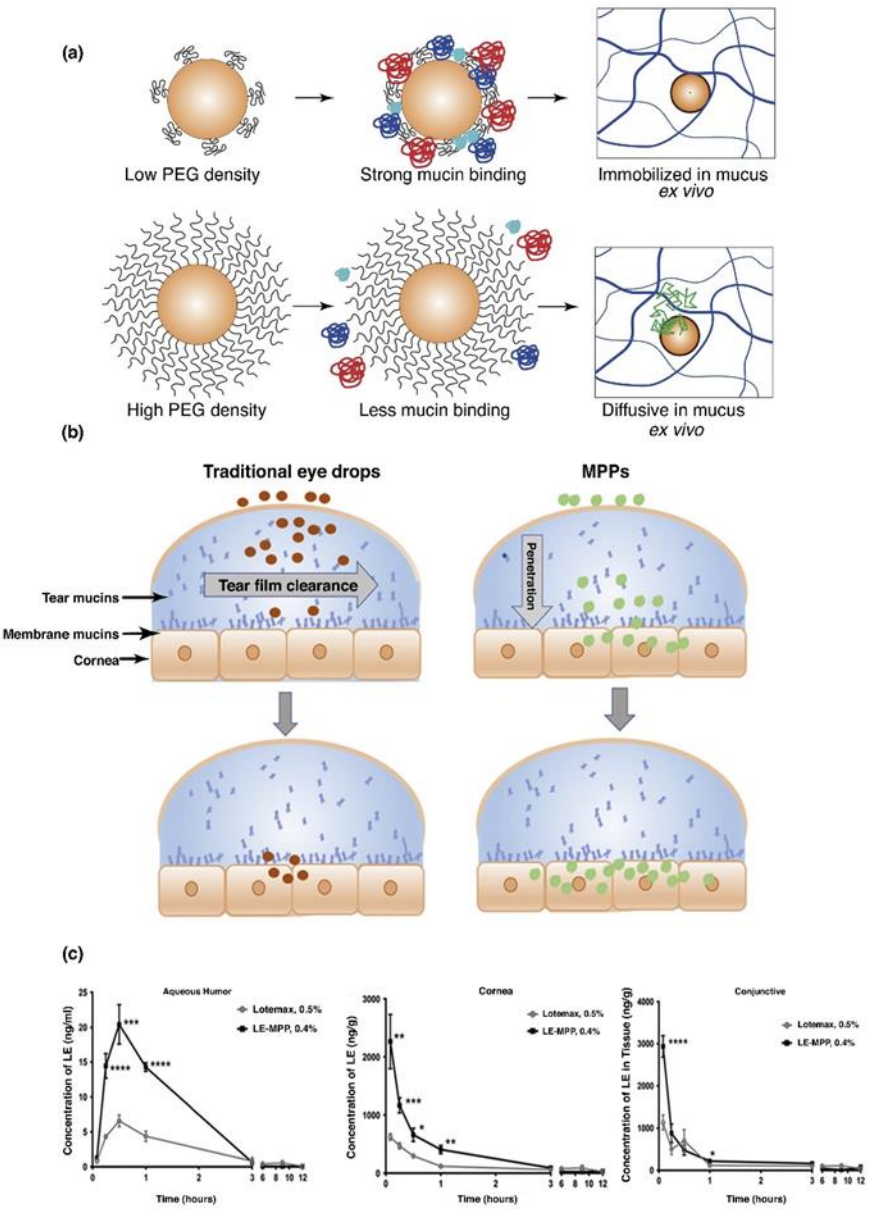


Figure 2.4. PLGA-PEG nanoparticles, demonstrating greater mucin binding, employing lower density PEG coating compared to higher density, which decreases adhesion on the surface of mucin of the polymeric system (**a**). *In vitro* evaluation of PLGA-PEG nanoparticles, illustrating rapid diffusion in mucus ex vivo (**b**). Comparative analysis of commercial loteprednol etabonate suspension eye drops to the polymeric delivery system in a New Zealand White (NZW) rabbit model, with almost 3-times greater Cmax profiles in rabbit aqueous humor, cornea and conjunctiva (**c**) (Reprinted with permission from (Meng et al., 2019).

2.5. Nanotechnology Employed in Ocular Drug Delivery

Nanotechnology, or more specifically, nanosystems, have been used more extensively in the medical field in recent years. It has a number of applications, including improving ocular drug delivery. These nanosystems have been shown to increase the bioavailability of ocular active ingredients; the primary challenge faced by conventional ocular treatments. The intrinsic design and make up of nanosystems

also allows for the protection of molecules, improved permeability through tissues and membranes and controlled drug release profiles (Du Toit et al., 2013).

Nanotechnology is defined as the development of structures and material which, in at least one dimension, fall within the nanometer scale (Weng et al., 2017).

One of the major benefits of employing nanosystems in ocular drug delivery are their ability to adhere to ocular tissue, mucosa and epithelium surrounding the eye; preventing formulations from being almost immediately washed away by the eye's defence mechanisms. This capacity for mucoadhesion has been studied since 1985, and since then, many different types of nanotechnology, ranging from nanoparticles to nanowires, administered through numerous methods, from topical application to intravitreal injections, have been investigated and developed in order to optimize and improve the delivery of drugs to the eye (Mohammed et al., 2019). The mucoadhesive property can be further advanced by the use of polymers in the formulation of the nanosystem. Increased mucoadhesive properties has been shown to increase the bioavailability of the active ingredient (Lorenzo-Veiga et al., 2019).

Until recently, nanosystems have largely been used to improve upon formulations which have already been developed and used on the commercial market. However, they have now been considered for formulations using active ingredients that are biologically active, but not able to be made into suitable formulations with conventional methods. Nanosystems can be used for targeted drug delivery as well as triggered release of active ingredients (Farokhzad et al., 2009).

It has been highlighted by Weng et al. that, although nano-sized ocular drug delivery systems have the potential to overcome the problems that are faced by current commercial products, there is still research that needs to be done in order to fully optimize these systems. For example, many of the studies done on nano-sized drug delivery have included *in vitro* studies and not *in vivo*. Those studies that do include *in vivo*, employ a rabbit model, as their eye closely emulates those of humans. However, there are differences between the two, especially regarding surface sensitivity, mucus productions and tear production. Thus, these factors could lead to differential results when tested in humans (Weng et al., 2017).

When considering the safety of nanotechnology for ocular drug delivery, it is fundamental to consider the material that the nanoparticles are composed of, thus playing a crucial role in whether the system

will cause irritation to the eye or not (Zorzi et al., 2011). This also applies to the chemicals that are used during the formulation of the system. For example, in a study by Leonardi et al., it was shown that the surfactants largely employed as stabilizing agents in nanosystems cause irritation to the eye. This study showed that some surfactants caused more irritation to the eye than others and some only caused irritation when they were used above a certain concentration. Their results showed that Kolliphor® P188 showed no irritation up to the highest concentration that was tested, Tween® 80 did not cause irritation up to a concentration of 0.05% and sodium dodecyl sulphate caused severe inflammation. This study highlights the importance of both the removal of excess surfactant once the nanosystem has been formulated, as well as proper selection of a suitable surfactant (Leonardi et al., 2014).

There are many advancements being made in the use of nanotechnology in ocular drug delivery, which are aimed at reducing the challenges of low bioavailability. The following subsections will be aimed at providing applications of different nanosystems for improved delivery of drugs to the eye.

2.5.1. Nanogels

Nanogels are composed of polymers, either naturally occurring or synthetic, crosslinked to form hydrogel particles that are within the nanoscale. The selection of polymers employed, as well as the respective concentrations at which they are used, determines 14 of the characteristics of the resulting nanogel. These characteristics include, among others, the charge, hydrophilicity and softness (Soni et al., 2016; Rejinold et al., 2012).

Nanogels are able to be used for ocular drug delivery due to their high drug loading capacity. Due to the gelling nature and large surface area, they are able to adhere to the mucosa surrounding the eye, allowing for improved delivery of ocular active ingredients (Brannigan et al., 2017).

These drug delivery systems are able to be administered to the eye in the form of drops, making them a suitable patient-friendly treatment option (Mohammed et al., 2019). This is due to the fact that nanogels are able to be engineered as *in situ* gels, whereby they can form a gel after application to the eye, as a response to a stimulus (Sivaram et al., 2015; Liu et al., 2016).

A study was performed by Liu et al., where an *in situ* nanogel system was developed for the delivery of curcumin, a compound which has low solubility and poor bioavailbility profiles when administered to the eye. The study showed that the nanosystem, compromised of curcumin cationic nanostructured

lipid carriers within a nanogel, had an increased mean residence time within the aqueous humor and improved cornea permeation than that of a curcumin solution (Liu et al., 2016).

When considering the use of nanotechnology in ocular drug delivery, the advancements that are being made aim to reduce, if not eradicate, the challenges faced by currently available ocular formulations.

2.5.2. Nanoparticles

Nanoparticles have a wide variety of uses, across a number of fields. In respect to the medical field, they have been extensively investigated and developed to aid in both diagnosis and treatment of diseases. This is largely due to the ability of nanoparticles to be engineered and subsequently functionalized in order to suit the needs of the system into which they will be placed (Zhao et al., 2018; Iglesias et al., 2018). There are multiple benefits in the use of nanoparticles for ocular drug delivery; for example, the nanometer scale allows for increased permeability across the blood-aqueous barrier. They also allow better drug stability in the formulation and sustained drug delivery (Janagam et al., 2017).

Nanoparticles are able to deliver drugs either by having the active ingredient adsorbed or encapsulated on the surface, or by having it incorporated into the particle itself (Zhao et al., 2018).

The figure below (Figure 5) demonstrates how chitosan is able to form nanoparticles to aid the delivery of hydrophobic drugs in a hydrophilic environment. This is an example of how nanoparticles are able to protect drugs from the solution or environment into which they are placed (Quinones et al., 2018).

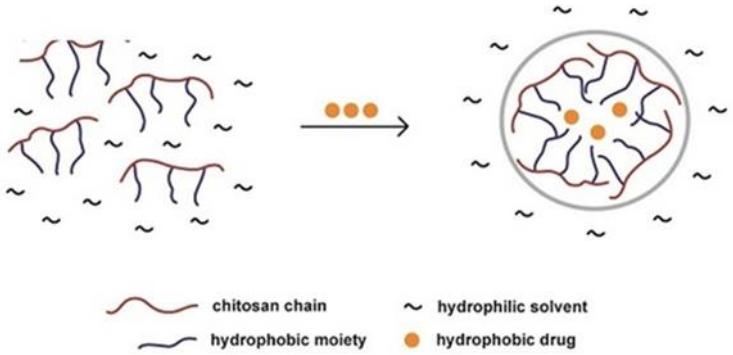


Figure 2.5. Illustration of hydrophobic drug entrapment within a chitosan nanoparticle, within a hydrophilic solvent environment (Quinones et al., 2018).

In order for nanoparticles to be administered topically to the eye, they need to be incorporated into a suspension system. This system is often designed with a hydrogel as a base (Abrego et al., 2015). Thus, while nanoparticles aid in the permeation of the drug through the barriers of the eye, the polymeric hydrogel allows for increased mucoadhesion to aid in greater bioavailability. A system such as this has been developed with the incorporation of levofloxacin, which showed increased corneal residence time as well as increased antibacterial activity when compared to that of a levofloxacin solution (Ameeduzzafar et al., 2018).

A study was performed by Abrego et al., whereby pranoprofen was incorporated into polymeric nanoparticles dispersed within a hydrogel. PLGA was used as the polymer to formulate the nanoparticles and Carbomer 934 (a polyacrylic acid) was selected as the hydrogel. The resulting formulations were compared against a free drug pranoprofen solution and commercial eye drops. The nanoparticle formulations showed prolonged *in vitro* drug release profiles in comparison to the other two formulations. Although the resulting formulations of this study did not show significant improvement in the corneal permeation of pranoprofen, it is worth noting that the nanoparticle system would be preferred, preventing irritation of the eye, in relation to the commercial eye drops, which are rapidly cleared from the eye after administration (Abrego et al., 2015).

Although the permeation of molecules into the posterior segment of the eye has been shown to be incredibly low, the development of nanoparticle containing systems, which are administered into the posterior segment of the eye through intravitreal or subretinal injections, will allow for increased mucoadhesion and sustained drug delivery. This in turn decreases the frequency that the injection needs to be administered, resulting in a decrease in side effects often associated, such as retinal detachment, hemorrhage or cataract development [Battaglia et al., 2016).

2.5.3. Nanosuspensions

Nanosuspensions are made of a drug dispersed through a colloidal carrier which is within the nanometer range. These systems are usually stabilized by the presence of a polymer or surfactant and allow for increased retention at the cornea as well as improved bioavailability of ocular active ingredients, such dexamethasone. They have also been shown to increase the antibacterial activity (Bachu et al., 2018).

Nanosuspensions have been considered as a possible delivery system for hydrophobic drugs. They allow for easy administration for the patient in the form of a drop, as well as the benefits of increased residence time and bioavailability (Patel et al., 2013; Das et al., 2011).

Polymeric nanosuspensions have been investigated for the delivery of ocular active ingredients because they harness the biocompatibility benefit of polymers, meaning they can be applied to the eye topically without causing irritation to the cornea (Sahoo et al., 2008). A variety of polymers, such as PCL and PLGA, have been investigated for inclusion in a nanosuspension drug delivery system as they are easy to prepare (Shi et al., 2015). These drug delivery systems also offer a prolonged drug release profile, compared to that of an aqueous solution (Pignatello et al., 2002).

This property of nanosuspensions was demonstrated in a study conducted by Pignatello et al. The researchers formulated a nanosuspension designed for the intraocular delivery of ibuprofen. The formulation was comprised of a Eudragit RS100® nanoparticulate suspension. *In vivo* studies showed that the concentration of ibuprofen measured in the aqueous humor was significantly higher in the rabbits treated with the nanoparticulate suspensions, than in those treated with an ibuprofen solution of the same concentration ($1.54 \pm 0.06\ \mu \cdot mL^{-1}$ compared to $0.93 \pm 0.08\ \mu \cdot mL^{-1}$ respectively). This was attributed to the fact that the sustained drug release profile, which was observed in the nanoparticulate suspension, allowed for better penetration through the ocular barriers, ensuring that a higher concentration of drug reaches the anterior chamber (Pignatello et al., 2002).

2.5.4. Nanomicelles

Nanomicelles are a form of nanotechnology composed of amphiphilic monomers. These polymers are able to self-assemble into micelles ranging from 20–200 nm. The ability of a polymer to self-assemble into micelles is dependent on its concentration within the solution. The critical micelle concentration must be reached in order for micelles to form (Vaishya et al., 2014). The amphiphilic nature of the polymers arranges with a hydrophobic core and a hydrophilic outer layer. This allows for the encapsulation and transport of poorly water-soluble molecules (Cagel et al., 2017; Cholkar et al., 2012).

Polymeric micelles, when used in ocular drug delivery, are able to encapsulate the active ingredient and deliver it to the appropriate target site. This helps to alleviate some of the localized adverse side effects that are experienced when using eye drops, such as dry eyes, burning sensations or stinging (Cagel et al., 2017). It also protects the active ingredient from degradation and increases its

permeation through the epithelial layers (Vadlapudi et al., 2014). Polymeric nanomicelles also offer benefits in terms of preparation and formulation, having a low critical micelle concentration and being stable in solution (Vaishya et al., 2014).

Nanomicelles, while providing a number of benefits for formulations which treat the anterior segment of the eye, have also been investigated to aid in drug delivery to the posterior segment, without the need for invasive injections. This has been shown to be possible in the delivery of rapamycin to the posterior segment of the eye after topical administration (Cholkar et al., 2015).

A nanomicellar formulation was developed by Cholkar et al. and studied from the delivery of rapamycin to the posterior segment of the eye. Rapamycin is hydrophobic, poorly soluble and is both a pH and light sensitive drug. The development of a nanomicellar fornulation was designed to assist in overcoming these challenges through a non-invasive delivery route. A blend of materials was employed; namely tocopherol, PEG succinate-1000, octoxynol-40 (with a viscosity enhancer), as well as povidone K 90; employed to overcome the rapid drainage of the formulation following topical application. An *in vivo* distribution study was performed which illustrated that the formulation was able to deliver rapamycin to the posterior segment of the eye after it had been applied topically. The results of the study showed that rapamycin was retained within the retina-choroid at a concentration of 362.35 ± 56.7 ng/g of tissue. This research provides a possible method of back-of-the-eye drug delivery, without using invasive procedures such as intravitreal injections (Cholkar et al., 2015).

2.5.5. Nanofibers

Nanofibers are made through one of two processes, electrospinning or sol-gel process. They offer an advantage of a large surface area (nanofibers with a diameter of 100 nm can offer a surface area of up to 1000 square meters per gram). This allows for increased drug loading abilities (Deepak et al., 2018; Sun et al., 2016). This, coupled with the sustained release profiles of biodegradable polymers as discussed earlier, may provide a new innovation in the treatment of posterior section conditions, such as AMD. The process of electrospinning allows for the easy incorporation of active ingredients into the system, allowing for increased drug loading within the nanofibers (Kovacs et al., 2017). This process of producing nanofibers also forms a high porous network of fibers (Da Silva et al., 2015; Sun, et al., 2016).

In a recent study by Lancina et al., nanofibers were loaded with brimonidine tartrate and investigated as a possible new topically administered formulation for the treatment of glaucoma. Polyamidoamine dendrimers were used for the formulation of the nanofibers. When evaluating the *in vivo* efficacy of this formulation, researchers found that the dendrimer nanofiber formulation did not cause a drop in intraocular pressure (IOP) significantly different to that of saline eye drops when administered as a single dose. However, when administered over a three-week period, the nanofiber formulation resulted in significantly lower pressure values. This highlights that while the nanofiber formulation has a similar efficacy to conventional eye drops, they have an additive effect over a longer time period. (Lancina et al., 2017).

2.5.6. Nanoliposomes

Nanoliposomes (including solid lipid nanoparticles and nanostructured lipid carriers) offer some interesting benefits in terms of ocular drug delivery. In addition to improving corneal permeability and residence time, they are able to withstand autoclave sterilization and can be loaded with poorly water-soluble drugs. This allows for the development of formulations that contain active ingredients which have a low bioavailability when administered as a suspension, such as penicillin G (Gomes-Ballesteros et al., 2019). Liposomes, with a lipid outer layer and an aqueous core, are able to be loaded with either lipophilic active (in the outer layer), hydrophilic (in the core) or amphiphilic active ingredients (Agarwal et al., 2016; Wang et al., 2018).

Nanoliposomes have also been shown to slow the rate of clearance of the formulation. They have been investigated for drug delivery to both the anterior and posterior segments of the eye. An important consideration for a drug delivery system that contains nanoliposomes is that it is prepared as a homogenous formulation to prevent the formation of agglomerates which could cloud the vitreous (Kaiser et al., 2013). A recent study developed nanoliposomes in a formulation for the treatment of dry eye disease. The incorporation of nanoliposomes allowed for the formulation to more closely resemble the make-up of the natural tears (Vicario-de-la-Torre et al., 2018).

In a recent study by Wang et al., nanoliposomes were investigated for the ocular delivery of brinzolamide (an anti-glaucoma active ingredient), as a complex with hydropropyl-β-cyclodextrin. This drug delivery system was formulated and compared to a commercially available brinzolamide suspension. While the nanoliposome formulation displayed only a moderate sustained release profile, it showed a 9.36-fold increase in the permeability coefficient. The nanoliposome formulation (with a brinzolamide concentration of 1 mg/mL) also showed an improved IOP reduction in comparison to the

commercial product (with a brinzolamide concentration of 10 mh/mL). Over an extended period of time the nanoliposome formulation maintained an effective IOP until the 12th hour, whereas the suspension reached its peak IOP reduction at 1 hour (Wang et al., 2018).

2.5.7. Nanowires

Nanowires are structures that have a diameter within the nanoscale range but are also able to have an elongated length. This allows for increased drug loading over other nanostructures such as nanoparticles (Pondman et al., 2015). They have often been combined with drug particles in drug delivery systems which were then able to adhere to epithelial tissues (Delcassian et al., 2018). Nanowires are able to offer controlled drug release profiles (Tian et al., 2012). This property would be beneficial in the delivery of drugs to the eye as it will reduce the frequency at which formulations, such as intravitreal injections, need to be administered.

In a recent study by Christiansen et al., PCL short nanowires, PCL electrospun and PCL smooth scaffolds, were compared for the delivery of retinal progenitor cells (RPCs). RPCs are used as a restorative procedure for retinal degenerative conditions, due to retinal cells not being able to regenerate. The PCL short nanowires provided a delivery system that displayed appropriate stiffness and flexibility, was able to be inserted into the retina effectively, as well as being flexible enough not to distort the shape of the subretinal space. The nanowires also provided the highest preservation rate of the overlying retina when compared to the other two scaffolds (17% higher than the PCL electrospun and 25% greater than the PCL smooth scaffold system) (Christiansen et al., 2012).

2.6. Future Developments in Ocular Drug Delivery Using Biodegradable Polymers and Nanotechnology

As of 2018, there were 51 nanotechnology medical products on the market, as reported by Patra et al. The researchers also reported a number of nanotechnology-based systems that are in the stage of clinical trials. This shows that the development of nanotechnology is on the rise (Patra et al., 2018).

Although polymer-based drug delivery has been the focus of much research in recent years, there are still advances to be made in order to fully enhance the beneficial properties that these materials possess. For example, further investigation into stimuli-responsive polymers could provide many new opportunities for improved ocular drug delivery systems (Mahlumba et al., 2016). Figure 6 shows possible administration routes for *in situ* forming gelling systems to the eye.

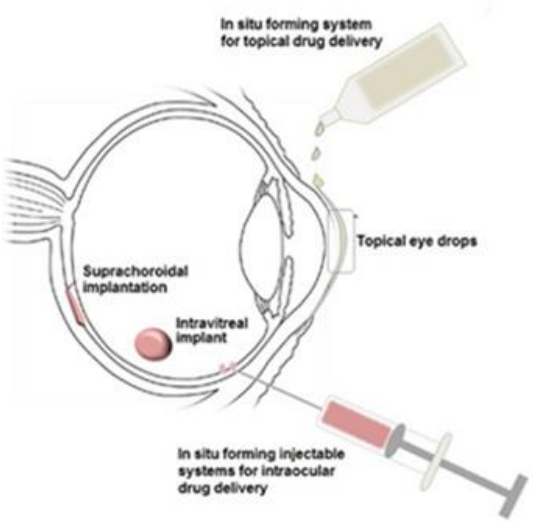


Figure 2.6. Illustration of *in situ* gelling systems that could be delivered to the eye employing stimuli-responsive polymers as biodegradable drug delivery platforms (Mahlumba et al., 2016).

Both polymeric drug delivery systems and nanotechnology can be employed in future research for the delivery of larger molecules, such as proteins and peptides, to the eye (Mahlumba et al., 2016).

Many of the studies that were mentioned in this review stated that, although the formulations developed were successful in terms of *in vitro* and *in vivo* studies, they require further investigations and clinical trials in order to be commercially viable. It can also be noted that some formulations, such as nanoparticles and hydrogels, are more extensively researched than others, such as nanowires. These can be attributed to research facility limitations, as well as high costs of undertaking such studies. Thus, further research should be done into these less investigated drug delivery systems, providing greater expansion to the field of biodegradable polymeric nanosystems for ocular drug delivery.

2.7. Conclusion

The importance of a patient's sight cannot be overstated, and there is a myriad of conditions that places this valuable sense in jeopardy. Although there are many ophthalmic formulations available on today's market, the drawbacks that they entail, such as low bioavailability and rapid clearance from the eye, leads to below optimum treatment plans for the patient. Patients either have to administer treatment at a regular basis, which puts them at risk for side effects, or undergo frequent, invasive procedures. Biodegradable polymers, together with nanosystems, offer many exciting opportunities

in terms of ocular drug delivery. Not only do they provide mechanisms in order to optimize the products currently on the market, they also allow for the development of many new formulations. The polymers that have been highlighted in this article have been shown to be biocompatible, biodegradable and mucoadhesive; all properties that are vital in overcoming the challenges faced by ocular drug delivery. Nanotechnology is rapidly emerging in the field of drug delivery. It has been widely used for diagnosis and treatment in other areas of the body. The advancements that are being made in this field provide a range of drug delivery designs, each offering a unique set of benefits. Innovations are being made in the field of ophthalmic drug delivery, as well as how both polymers and nanosystems can be used in further formulation developments. Thus, innovations made in terms of polymeric nanosystem drug delivery are vital for overcoming the challenges faced in treating ocular conditions.

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CHAPTER 3

HYDROGEL BIOMATERIALS FOR APPLICATION IN OCULAR DRUG DELIVERY

3.1. Introduction

There have been many recent advancements made in the delivery of drugs to the eye, a site that is challenging to treat. The eye is a relatively isolated organ within the body, with many barriers and mechanisms that limit the entry of foreign substances into the eye. These include, among others, the cornea, blinking reflex, blood-aqueous barrier, blood-retina barrier and the nasolacrimal drainage system. Collectively, these systems make the delivery of drugs to both the anterior and posterior segment of the eye more difficult (Patel et al., 2013). Novel drug delivery systems are constantly being developed to overcome the low bioavailability observed in many conventional ophthalmic formulations; these novel systems include the development of hydrogels.

Hydrogels have been largely investigated within the medical industry for a number of purposes, including drug delivery and tissue engineering. These systems are composed of cross-linked polymers which are capable of swelling when placed in water or an aqueous environment. Hydrogels have been researched in terms of drug delivery because they are able to hold, within the cross-linked matrix, a number of different substances. These range from hydrophobic and hydrophilic molecules to both micro- and macromolecules (Kang Derwent et al, 2008). An example of the effectiveness of hydrogels in drug delivery is shown in the article by Li et al. where the delivery of antibiotics by hydrogel systems was discussed. It was highlighted how hydrogels are able to deliver antibiotics to a local site (overcoming the severity of side effects often seen with systemic administration), offer controlled release of the active ingredient and have better biocompatibility than conventional drug delivery systems. (Li et al., 2018). These benefits can be translated into the development of hydrogel systems for the delivery of drugs to the eye.

Due to the fact that hydrogels are so versatile and are able to be modified to exploit the environment and function they are being designed for; these systems are highly advantageous in the effective delivery of drugs to the eye (Kang Derwent et al., 2008). Figure 1 below indicates the various potential applications for hydrogels in ocular drug delivery.

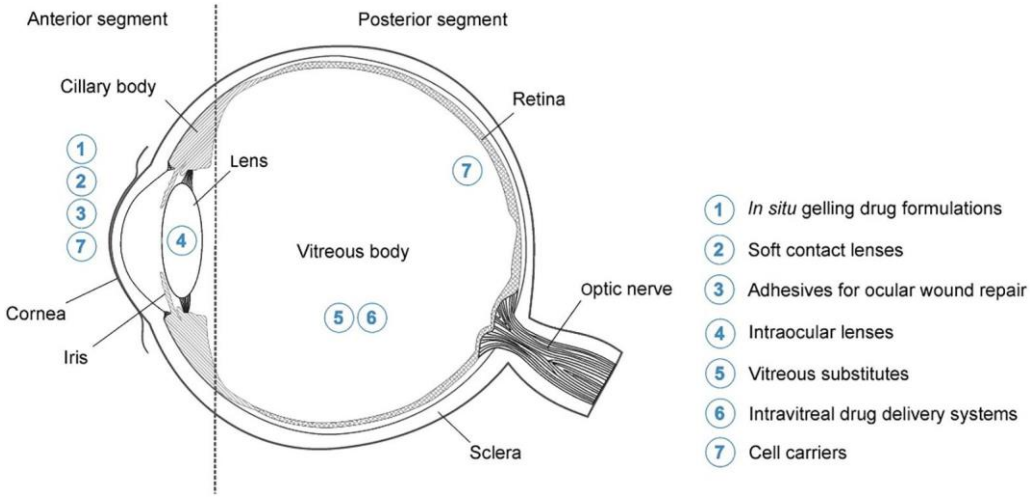


Figure 3.1. Highlighting the potential application for hydrogels in ocular drug delivery. These include the delivery of drugs to both the anterior and posterior segments of the eye which will aid in overcoming the physiological barriers. Possible topical formulations for delivery to the anterior segment include systems which gel upon application (*in situ* gelling formulations) and contact lenses. Posterior segment formulations include intravitreal injections, which are made more effective by hydrogel technology, and cell carrier systems (Kirchhof et al., 2015).

Hydrogels have been shown to alter the drug release profiles of a formulation (to a sustained drug release profile), largely due to the swelling rate and water adsorption properties of the biotechnology platform. This swelling rate of the hydrogel can be induced as a response to a change in the environment into which the hydrogel is placed; these are known as “smart” or stimuli-responsive hydrogels. The stimulus can be chemical or physical and allows for the development of drug delivery systems which are regulated by the body. In addition, these “smart” hydrogels are able to respond to external stimuli such as in the process of iontophoresis (Fathi et al., 2015).

Through the development of stimuli-responsive hydrogel systems, not only are researchers able to overcome the issues of low bioavailability and rapid removal from administration site, which is currently seen with conventional formulations, they are also able to do so without comprising on patient comfort. These delivery systems are able to be administered as a liquid and then form a gel once in contact with the eye (Hamcerencu et al., 2020). This is an important factor to consider in terms of patient compliance as patients are less likely to make use of an ophthalmic formulation if it is difficult to administer which is often the case with formulations that are highly viscous such as ointments (Singh et al., 2019).

Polymers have received much attention for use in drug delivery, and more specifically ocular drug delivery, over recent years. Although there are countless polymers available, this review article focuses on those which occur naturally, also known as biopolymers. These specific polymers offer the beneficial properties of being biodegradable, biocompatible and non-cytotoxic. They also have the advantages of being readily available, renewable and less expensive in comparison to synthetic polymers (Oh et al., 2009).

3.2. Physiological Ocular Barriers and Defense Systems Which Impact Drug Delivery

There are many challenges when it comes to effective delivery of drugs to the eye. Many of these are as a result of the barriers and mechanisms present within the eye which are designed to protect it from foreign particles and substances. A brief overview of the major ocular defense mechanisms is discussed below.

The first defense mechanism found in the eye are pre-corneal factors which result in the low bioavailability of topically applied ocular formulations. These include the blinking reflex, high tear turnover rate and the lacrimal drainage of the solution. The cul-de-sac of the eye can hold approximately 30 µl of an administered eye drop. However, majority of this is removed within 15 – 30 seconds after the drops have been administered (Gaudana et al., 2010). Considering these factors, drug delivery systems need to be developed that are able to improve the retention of the formulation at the administration site. Consequently, this will improve the penetration of the active ingredient into the eye. Both hydrogel systems and mucoadhesive biopolymers could furnish formulations with these much-needed advantages.

One of the major barriers to foreign substance entry into the eye is the multiple layers through which substances must pass through in order to penetrate into the target tissues. These layers include the cornea and the conjunctiva, among others. The cornea is located in the anterior segment of the eye and it made up of 6 layers: the epithelium, Bowman's membrane, stroma, Dua's layer, Descemet's membrane and the endothelium (Ludwig et al., 2005; Dua et al., 2013). It is one of the main penetration-limiting layers in terms of drug delivery. This layer is highly lipophilic which largely prevents the entry of hydrophilic molecules into the eye (Moiseev et al., 2019).

The conjunctiva is a highly vascularized membrane that covers most of the anterior aspect of the eye. This high vascularity means that, although it can be used for the delivery of hydrophilic and large

molecules, a large portion of the administered drug will be removed via the conjunctiva and enter systemic circulation before penetrating into the eye. This is also one of the main reasons why topically administered drugs are not able to reach the posterior segment of the eye in effective concentrations (Willoughby et al., 2010).

The eye is composed of two segments; the anterior segment (composed of the aqueous humor, conjunctiva, cornea, iris, ciliary body and lens) and the posterior segment (composed of the choroid, optic nerve, retina, sclera, choroid and vitreous humor). Each segment is susceptible to a range of conditions and each poses its own challenges when it comes to drug delivery (Souto et al., 2019). There are two blood-ocular barriers: the blood-aqueous barrier and the blood-retinal barrier. These largely prevent the entry of substances into the eye from systemic circulation. Although systemic administration has been considered as a route for drugs needed in the posterior segment of the eye, the dose needed is often high which leads to unwanted side effects (Nettey et al., 2016). Figure 2 highlights the blood-ocular barriers in addition to the tissues which comprise these barriers.

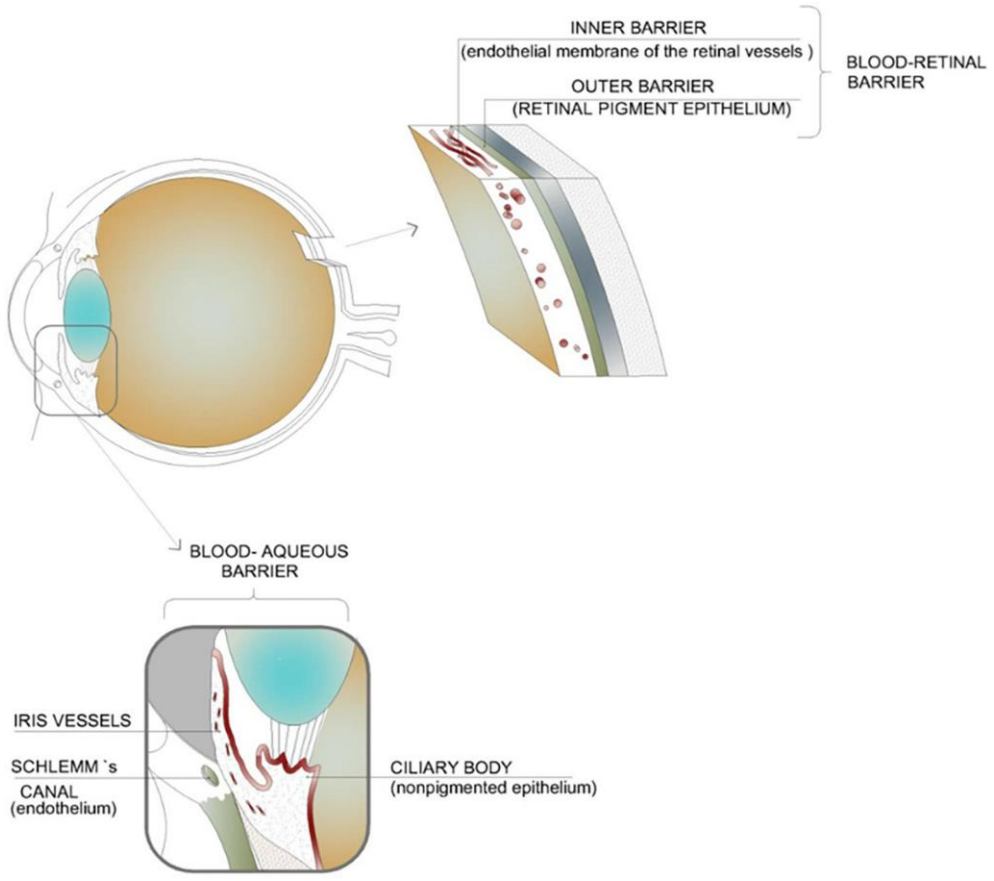


Figure 3.2. Illustration of the blood-ocular barriers which inhibit the movement of active ingredients into the eye from systemic circulation; namely, the blood-aqueous barrier and the blood-retinal barrier. These barriers result in the need for high systemic dosages of drugs in order to achieve an adequate concentration within the intended tissues. This high dosage can lead to unwanted side effects (Occhiutto et al., 2012).

When a formulation is applied to the surface of the eye (i.e., topical administration), it is rapidly removed through the blinking reflex and nasolacrimal drainage. This drainage system removes the drug from the eye via the nasolacrimal duct. It then enters the nose and is absorbed by the nasal mucosa where it enters into systemic circulation. This is another factor which furthers the low bioavailability of topical applied ophthalmic preparations (Rajasekaran et al., 2010).

Hydrogels have been shown to increase the residence time of an active ingredient, allowing more time for it to diffuse through the layers of the eye. This plays a major role by increasing the bioavailability of topically administered ophthalmic formulations (Vashist et al., 2014). Due to the increased viscosity of a hydrogel system, it is also better able to withstand the clearance of the formulation due to blinking, further improving the bioavailability (Li et al., 2013).

Biopolymers have also been shown to help overcome these barriers to drug delivery. Some, such as chitosan, have inherent mucoadhesive properties which allows the formulation to remain at the administered site for a longer period of time. (Fulgencio et al., 2012) Cellulose derivatives have also been used to enhance the viscosity of a formulation, thereby preventing it from being washed away from the ocular surface too rapidly (Rajasekaran et al., 2010).

3.3. Current Commercial Formulations Utilized for the Delivery of Drugs to the Eye

There are many formulations currently on the market which are designed to treat ophthalmic conditions. These range from anterior segment conditions such glaucoma, bacterial conjunctivitis and post-operative inflammation to posterior segment conditions such as neovascular age-related macular degeneration (AMD), uveitis and macular oedema (Sultana et al., 2006; Bao et al., 2017; Kaji et al., 2018). Each of the drug delivery systems discussed below has distinctive disadvantages when it comes to the effective delivery of drugs to the eye. It has been shown that the inclusion of hydrogels into the drug delivery system has been able to overcome some of these challenges, as is highlighted by the various studies included below.

Currently, the most common dosage form used to treat ocular conditions is eye drops. These formulations can be solutions or suspensions. However, although they are the first line treatment, there are many limitations to their use. These range from low bioavailability and rapid clearance from the administration site to poor patient compliance. (Yellepeddi et al., 2016) Active ingredients in eye drops are not able to penetrate through to the posterior segment of the eye and thus are mainly used to treat anterior segment conditions (Urtti et al., 2006).

Conventional, commercially available eye drops often have frequent dosing schedules (ranging from daily to multiple times a day) and, in the case of chronic conditions such as glaucoma, require the patient to use them on a long-term basis. This can lead to unwanted side effects, which, for example, has been seen with latanoprost eye drops (daily administered dose of one drop). These side effects can cause patients to stop using their medications as prescribed, or to not use them at all. This is another reason why novel drug delivery systems such as hydrogels are needed; to reduce the frequency of dosing, reduce side effects and be patient-friendly enough so that patients will use them for an extended period of time if need be (Cheng et al., 2016).

In a recent article written by Yadav et al, it was highlighted how pre-corneal factors lead to the low absorption of ocular active ingredients used to treat glaucoma, administered as eye drops. These factors, such as tear turnover rate and the drainage of the formulation from the administration site, result in a 70 – 80 percent loss of the amount of drug which is administered. It was also highlighted how the frequent dosing schedules of eye drops can cause damage of to the eye. The consideration of ointments has been made, as these formulations have a higher viscosity and are not as rapidly drained from the eye as a liquid formulation. However, ointments are known to cause blurred vision when administered which leads to poor patient compliance (Yadav et al., 2019).

Posterior segment conditions are generally treated using sub-tenon, intravitreal or systemic administration. However, each of these routes also comes with challenges of its own. One of the main objectives in the development of new drug delivery systems for the posterior segment is to reduce the invasiveness of the formulations which are currently used. For example, anti-vascular endothelial growth factors (anti-VEGF) are used to treat a number of posterior segment conditions, namely those affecting the retina such as myopic choroidal neovascularization and diabetic macular oedema. However, anti-VEGF is currently only able to be administered via intravitreal injections as the molecule are large and hydrophilic which prevents them from penetrating through the various barriers. This highlights the need for new technologies and drug delivery systems which are able to deliver molecules such as anti-VEGF without frequent, invasive injections (Wong and Wong, 2019).

Intravitreal injections are able to deliver a high concentration of the drug directly into the vitreous of the eye but are invasive and pose risks such as retinal detachment, vitreous hemorrhage and endophthalmitis. The chances of these happening increases with the frequency of administration (Urtti et al., 2006; Gaudana et al, 2009). The use of hydrogels as intravitreal injections, with their extended drug release profiles, can delay the frequency of intravitreal injections, thus lowering the

chances of the aforementioned risks occurring. Table 1 highlights the formulations which are currently used to treat ophthalmic conditions, both in the anterior and posterior segment of the eye. A brief breakdown of the disadvantages of each of the formulations is also given.

Table 3.1. Current ophthalmic formulations which are used to treat anterior and posterior segment conditions. These formulations, both topical and intraocular, each have a number of disadvantages or challenges in terms of drug delivery which can be overcome by hydrogel systems.

Administration	Preparations	Conditions	Disadvantages	References
Topical preparations	Eye drops (solutions and suspensions)	Glaucoma, Dry eye, infectious keratitis, conjunctivitis anterior uveitis, post-operative inflammation.	Low bioavailability, frequent dosing regimen, preservatives often used in formulation.	Sultana et al., 2006; Gupta et al., 2013
	Ointments and gels	Open-angle glaucoma, Dry eye, Blepharitis bacterial conjunctivitis.	Poor content uniformity, Known to cause blurred vision when applied, inaccurate dosing, eyelid matting.	Bao et al., 2017; Li et al., 2013; Shen e al., 2018.
	Contact lenses	Post-operative barrier for protection of cornea, pain relief, protection of cornea following injury.	Lack controlled release mechanism, drug is released from the system very quickly.	Lim et al., 2001; Tieppo et al., 2012.
Intraocular preparations	Intravitreal injections	Neovascular AMD, diabetic macular oedema, prolifertive diabetic retinopathy choroidal neovascularization.	Invasive procedure for the patient, possible complications (retinal detachment, endophthalmitis, subconjunctival haemorrhage and cataract formation)	Kaji et al., 2018.
	Subtenon injections	Macular oedema, intermediate uveitis.		Thomas et al., 2006; Ozdek et al., 2006;

			Active ingredient must cross multiple barriers before reaching the retina, occasionally less effective than intravitreal injections,	Bonfioli et al., 2005.
	Intraocular implants	Uveitis, cytomegalovirus retinitis, diabetic macular oedema.	Invasive surgical insertion and removal (if the implant is not biodegradable), predetermined drug release rates	Yasin, 2014; Wang et al., 2013.

3.4. Characterization Between Physically and Chemically Cross-Linked Biotechnology Hydrogel Systems

As previously mentioned, hydrogels are formed from polymers through a process known as cross-linking. Cross-linking occurs when one polymer chain is linked to another chain via a bond, either through a chemical or physical process. It is these bonds which give hydrogels their stability and multidimensional network structure. The process of cross-linking a hydrogel can have an impact on its physical properties such as elasticity, viscosity and solubility (Maitra and Shulka, 2014).

Although chemical and physical cross-linking methods each have their own advantages and disadvantages, it is worth noting that physically cross-linked hydrogels do not employ agents containing reactive functional groups which may cause inflammatory responses *in vivo.* However, these hydrogels also result in limited control over how the hydrogel is degraded within the body and, if the physical bonds are not strong enough, the inevitable dilution within the body can negatively impact the mechanical integrity of the hydrogel (Patenaude et al., 2014).

3.4.1. Hydrogels Which are Cross-Linked Through Physical Bonds

Physical bonding occurs through interactions between the polymer chains such as ionic bonding, Van der Waals forces, hydrogen bonding or hydrophobic forces. Due to these types of bonds, the hydrogels formed through physical bonds are known to be reversible and have a degree of instability (Trombino et al., 2019). The hydrogels formed through physical interactions are generally less stable than those formed through chemical interaction as these bonds are susceptible to formation and breakage when there are changes in pH, temperature and ionic strength. However, this can be a favourable characteristic if the desired outcome is a reversible hydrogel (Kirchhof et al., 2015).

3.4.2. Hydrogels Which are Cross-Linked Through Chemical Bonds

Chemically formed hydrogels are known as "permanent" hydrogels due to the covalent bonds which form between polymer chains. These systems allow more stability and maintain their structure better than the physical hydrogels (Trombino et al., 2019). However, it is important that the cross-linking agent can be removed completely from the hydrogel, or a non-toxic agent is used so as to prevent adverse tissue reactions when the hydrogel is placed into the eye (Hoare and Kohane et al., 2008).

The stability of a chemically cross-linked hydrogel was demonstrated by Yu et al. In this study a hydrogel comprised of hyaluronic acid and dextran was evaluated for the delivery of bevacizumab, a monoclonal antibody which is used to treat neovascular diseases (Grisanti and Ziemssen, 2007). The hydrogel system was designed so that once it had been injected intravitreally, the polymers would form a solid gel. While this delivery system design has the benefits of a chemically cross-linked hydrogel, it also does not contain any cross-linking agent (the polymers cross-link with each other in response to physiological conditions) thereby improving its biocompatibility. The hydrogel system was able to release the active ingredient via a controlled release mechanism and maintain a therapeutically relevant concentration within the vitreous over a period of six months during *in vivo* studies. This would eliminate the current monthly schedule needed for bevacizumab administration, the risks of which have been discussed above (Yu, et al, 2015).

3.5. Stimuli-Responsive and *in Situ* Hydrogel Systems and Their Applications in Ocular Drug Delivery

In situ forming gel preparations offer an interesting advancement in sustained drug release profiles. This can be particularly useful in terms of the delivery of drugs to the eye as these systems provide an increased retention time at the cornea as well as prevent the rapid removal of the formulation via the nasolacrimal drainage system (Cheng et al., 2016). Both of these factors play a role in overcoming the current challenge of low bioavailability seen in many ocular drug delivery preparations.

These *in situ* gelling systems are a type of stimuli-responsive hydrogels that are able to be administered to the eye as a liquid drop and subsequently form a gel after administration; known as a sol-gel transition. Gelation can be brought about as a response to a change in pH, ionic content or temperature; although not all hydrogel systems are designed as stimuli-resonsive systems and are simply administered as a gel (Al Khateb et al., 2016). Along with the ease of administration and prolonged retention time*, in situ* gelling systems have other advantages such as accurate dosing, simple formulation processes and easy sterilization (Agrawal e al., 2010). Figure 3 depicts the various stimuli which can cause a hydrogel to swell or de-swell.

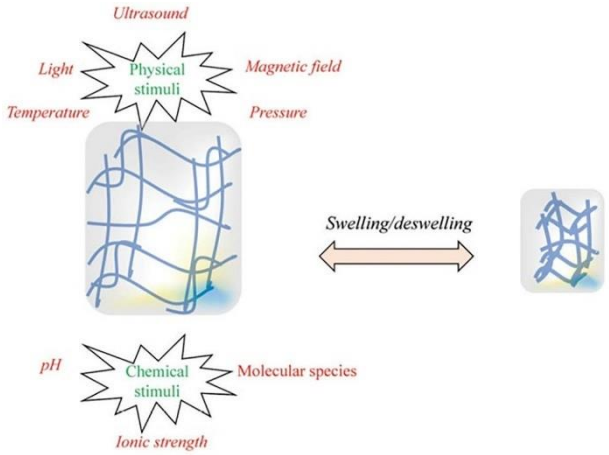


Figure 3.3. Illustration of the chemical and physical stimuli to which a hydrogel can respond. These stimuli are able to be provided by the body (for example, temperature and/or pH changes between conditions under which the hydrogels are stored and the conditions of the site into which it is administered) or externally (for example, ultrasound waves or a magnetic field). These stimuli can cause or a hydrogel to swell or de-swell, depending on how the formulation is designed. Reversible hydrogels are able to return to their original state when the stimulus is removed (Fathi et al., 2015).

In situ gelling systems have also been shown to exhibit sustained drug release profiles, another beneficial factor in ophthalmic drug delivery. This has been observed in many of the studies which are discussed below.

3.5.1. Temperature-Sensitive Hydrogel Systems

Temperature-sensitive, also known as thermosensitive, hydrogels undergo swelling or de-swelling in response to a change in temperature. There are 3 classifications of thermosensitive hydrogels; negatively thermosensitive (these contract in response to an increase in temperature), positively thermosensitive (these contract in response to a decrease in temperature) and thermally reversible gels (Masteikova et al., 2003).

Thermosensitive *in situ* hydrogels, which are commonly utilized for drug delivery purposes are liquid at room temperature (20 - 25°C) and form viscous gels at body temperature (34 - 37°C). The polymers used in these systems have a lower critical solvent temperature; the temperature at which the sol-gel transition occurs. It is important that this critical temperature is close to bodily temperatures so that the systems does not require an external heat source to form a gel (Kumar et al., 2013). The thermosenstive properties of these hydrogels have also be proven to be beneficial in recent cartilage tissue engineering research as they allow for minimally invasive administration yet form a scaffold with suitable mechanical strength. These systems are also able to mold into the irregular shaped area into which they are administered (Wang et al., 2019).

An *in situ* thermosensitive hydrogel was developed by Chen, X et al for the delivery of a model drug, levocitirizine dihydrochloride. The hydrogel system was comprised of chitosan and disodium α-d-glucose 1-phosphate (DGP) and showed many favorable results. The formulation was a low viscosity liquid at room temperature and a gel at physiological temperature. It showed an initial rapid release of the drug, followed by a sustained drug profile. When in a gel form, the system showed that it had a prolonged residency time, in comparison to that of an aqueous solution, as well as improved cornea penetration of the drug (Chen et al., 2012). This shows that a thermosensitive hydrogel system is able to overcome some of the challenges seen in conventional ophthalmic treatments.

3.5.2. pH-sensitive hydrogel systems

These *in situ* gelling systems either swell or de-swell as a response to a change in the pH within the environment into which it is placed. The polymers used in pH-sensitive hydrogels have ionic groups which give them their responsive ability. For example, cellulose acetate phthalate latex (formulation pH of 4.4) has been shown to form a viscous gel when placed into the cul-de-sac of the eye. However, the development of pH-sensitive gels must take into account the delicate environment of the eye. The formulation must have a buffer capacity that can form a gel when placed into the eye but not cause damage to the eye (Kushwaha et al., 2012).

Although many of the polymers used in pH-sensitive hydrogels are synthetic polymers, such as carbopol (poly acrylic acid) and poly (ethylene glycol) (PEG), natural biopolymers are also used in the formulations to give them more favorable characteristics (Kushwaha et al., 2012; Wu et al., 2013). For example, in a study performed by Kumar, S et al, it was shown that, although polyacrylic acid (PAA) is able to change from a low viscosity liquid when in an acidic solution to a gel at a higher pH, the amount of polyacrylic acid needed for this to occur was too high. This means that the solution could not be

neutralized by the tear fluid which acts as a buffer in the eye. To overcome this, hydroxymethylcellulose, a natural polymer also able to act as a viscosity modifier was added. Both the polyacrylic acid and the hydroxymethylcellulose were low viscosity liquids at pH 4.0 and transformed into viscous gels at a pH of 7.4. This meant that the concentration of PAA could be reduced to a safe level, without compromising the gelling and rheological behavior of the system (Kumar and Himmelstein, 1995).

The ability of methylcellulose, as hydroxypropylmethylcellulose, to act a viscosity modifier in a pH-sensitive gelling system was further demonstrated by Srividya et al. The researchers developed a pH-triggered *in situ* gelling system comprised of PAA and hydroxypropylmethylcellulose which was shown to be a viable system in the topical delivery of ofloxacin (Srividya et al., 2001).

3.5.3. Ion-sensitive Hydrogel Systems

An ion-sensitive gel transforms from a liquid to a gel as a result of a change in ion concentration within the environment it is exposed to. An example of such a gel is shown in a study by Liu, Z et al. The researchers formulated an alginate hydrogel for the delivery of gatifloxacin, a broad-spectrum antibiotic, which underwent a sol-gel transition when exposed to divalent cations. Methylcellulose was incorporated in order to decrease the amount of alginate needed for gelation. This formulation was able to release the active ingredient over an eight-hour period *in vitro* and formed a gel within the cul-de-sac of the eye when administered as a drop. This renders an ion-sensitive hydrogel a suitable alternative to conventional eye drops as it the increased residence time and sustained drug release profile will lead to an improved bioavailability (Liu et al., 2006).

3.5.4. Ultrasound-responsive hydrogel systems

Ultrasound responsive systems are able to deliver drugs to a specific site which prevents the side effects which can be seen with systemic administration of certain drugs. These systems can incorporate nanotechnology. Polymeric hydrogels or nanocarriers such as nanobubbles are loaded with the drug and, once administered, exposed to ultrasound waves. This then leads to cavitation and high temperatures at the site, causing the rupture of the polymeric chains of the nanobubble. (Mahlumba et al., 2016; Mura et al., 2013).

Ultrasound-responsive systems are able to deliver a drug at a rate which is controlled from an external source which make them particularly useful in the investigation of cancer treatment. An example is

the use of oxygen nanobubbles used for the delivery of mitomycin-C. The nanobubbles system was capable of lower tumor progression rates with a 50% lower drug concentration (Bhandari et al., 2018).

The application of ultrasound waves has been shown to be beneficial in the penetration of drugs through the various barriers of the eye, including the cornea. This was shown to be true in a study performed using dexamethasone where a significant increase in the permeability of the cornea was observed (Nabili et al., 2013). However, there is some concern over the increase in temperature which is induced as it may cause damage to the sensitive structures within the eye. A study was completed by Nabili et al which showed that the ultrasound frequency which had previously been shown to increase penetration was safe for the ocular tissues tested (Nabili et al., 2015).

3.5.5. Iontophoresis: An External Stimulus for More Effective Ocular Drug Delivery

Iontophoresis is a physical force-based response technique which is used to enhance the penetration of an ocular active ingredient through the various tissue layers found in the eye. This is done by applying an electric current between two electrodes; one which is used to deliver the drug and another which is placed on the body. The ionized drug is then able to travel through the tissue as a conductor of the current. Iontophoresis has been illustrated extensively in transdermal applications but has also been investigated for use in ocular drug delivery (Eljarrat-Binstock and Domb et al., 2006).

There are many challenges, which have highlighted throughout this article, associated with the delivery of drugs to the anterior chamber of the eye but there are even more challenges in the delivery to the posterior segment. Most active ingredients aren't able to penetrate through to the posterior segment when they are applied topically. This has led to the investigation of alternative routes of delivery such as intravitreal, subconjunctival or transscleral. Iontophoresis has also been considered to aid in delivering drugs to the posterior segment. This allows for the treatment of conditions such as retinitis, uveitis, diabetic retinopathy and AMD (Myles et al., 2005).

There are various device designs which can be utilized for iontophoresis; one such design includes a hydrogel. A hydrogel pad is saturated with a drug and acts as the delivery probe. This system has been shown to have promising results when tested with various drug entities such as dexamethasone. Transscleral hydrogel-based iontophoresis devices have been tested in both *in vivo* studies and clinical trials in healthy subjects and have shown good safety profiles as well as successful delivery of drug to the retina and choroid (Huang et al., 2018).

Although there are some iontophoresis devices which have been designed for transscleral drug delivery, the process does have some disadvantages. As with any medical procedure, there are risks involved; these include epithelial oedema, inflammation and burns (depending on the current density and duration of treatment). Iontophoresis has been demonstrated to be effective in improving the penetration of steroids, antibiotics and antivirals. However, it has been reported that it is not able to deliver macromolecules to the vitreous in rabbits at a significant concentration (Thrimawithana et al., 2011).

In a study by Eljarrat-Binstock et al, hydrogel iontophoresis was employed to deliver nanoparticles to the eyes in an *in vivo* rabbit model. This study also investigated whether positively or negatively charged fluorescence nanoparticles penetrated through the tissues better. The researchers noted that, while iontophoresis is effective in improving the penetration of drugs into the eye, each active ingredient needs to be evaluated separately due to the fact that the physiocochemical properties of the molecule will influence its behaviour during the procedure. In this study, the respectively charged nanoparticles were loaded into a hydrogel sponge and were administered via an iontophoretic device at the central cornea and at the sclera. After a specified amount of time the eyes of the rabbits were enucleated and tissue samples collected. The negatively charged particles showed penetration into the inner ocular tissues after 4 hours, which increased after 12 hours. However, the positively charged nanoparticles showed extensive penetration into the inner tissues at just 4 hours after administration, illustrating the effect of the physicochemical properties of the particles on their behaviour. Both of these indicate that iontophoresis is an effective way of ensuring the penetration of nanoparticles (which are able to be loaded with an active ingredient) through the eye (Eljarrat-Binstock et al., 2008).

Iontophoresis has also been used for the delivery of drugs through the suprachoroidal space. In a study performed by Jung et al., a micro-needle device was tested for the delivery of nanoparticles in an *ex vivo* rabbit model. The results showed that with an injection into the suprachoroidal space (SCS) without iontophoresis the nanoparticles that were localized around the site of injection (less than 15% delivered to the posterior region of the SCS). However, in the eyes on which iontophoresis was performed, over 30% of the nanoparticles were found in the posterior region of the SCS; this was also found in the *in vivo* study. This study shows how iontophoresis is able to improve the delivery of drugs to the eye and is able to be used in place of other delivery systems such as intravitreal injections (Jung et al., 2018).

3.6. Biopolymers Employed in the Formulation of Ocular Hydrogel Systems

Natural polymers have been widely investigated in a number of medical fields, including tissue engineering and drug delivery. This is largely due to the fact that they are biodegradable within the body and do not induce an inflammatory reaction (Singh, 2011). In terms of tissue engineering, they have also been shown to be conducive to cell growth and have a structure similar to the tissue matrix (Zhang et al., 2019). This section will focus on how natural polymers are employed in drug delivery systems.

These polymers, also known as biopolymers, have long been viewed as a crucial aspect in the developments that are achieved in the field of drug delivery. Highlighted below are biopolymers commonly used in ocular drug delivery systems. Their chemical structures are shown in Figure 4.

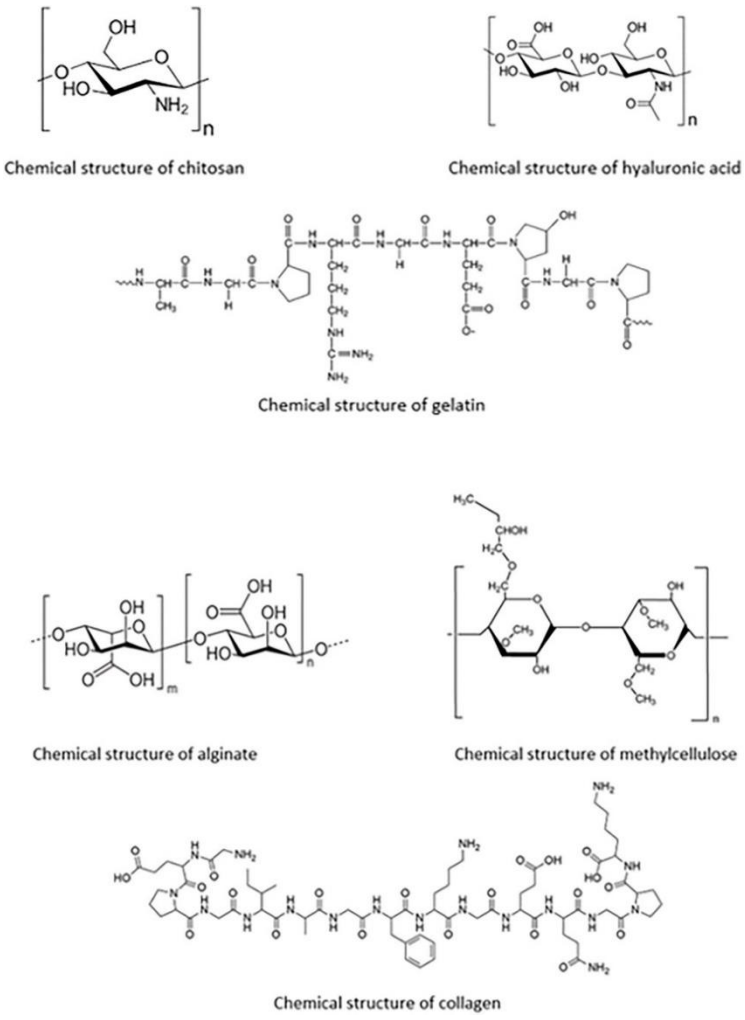


Figure 3.4. Chemical structures of each of the biopolymers; chitosan, hyaluronic acid, gelatin, alginate, methylcellulose and collagen, for ocular polymeric drug delivery.

3.6.1. Chitosan Polymeric Bio-Platforms

Chitosan is one of the most widely used polymers in polymeric drug delivery systems due to its biocompatibility, biodegradability and low toxicity profiles (Bhattarai et al., 2010). It is a cationic polysaccharide which is derived from chitin. One of chitosan's most beneficial qualities is its mucoadhesive properties. The mucoadhesion is due to the fact that the positively charged chitosan is able to interact with the negative charges found in mucin (Fulgencio et al., 2012). This quality allows for improved permeation of drugs through ocular tissues as well as their controlled release from the formulation; both of which are vital in improving the delivery of drugs to the eye (Duttagupta et al., 2015).

Although chitosan is a very useful biopolymer for drug delivery, it is only soluble in acidic solutions. This is not desirable, especially when it is being formulated in ophthalmic formulations. For this reason, chitosan is often modified, for example through PEGylation and carboxymethylation (Xu et al., 2013).

A thermosensitive chitosan-based hydrogel was formulated by Cheng et al. This system was designed to overcome some of the challenges seen with latanoprost eye drops such as unwanted side effects after long-term use and low bioavailability. The hydrogel was characterized using both *in vitro* and *in vivo* tests for drug release and biocompatibility. The system was shown to be well tolerated and non-cytotoxic. During the *in vivo* studies, using a rabbit model, latanoprost was found in the aqueous humor 7 days after a single topical administration of the system, suggesting that this system could be administered on a weekly base instead of a daily basis as the commercial product is currently (Cheng et al., 2016).

Chitosan is often used in combination with other natural or synthetic polymers. For example, a study was performed by Cao et al. where a poly(N-isopropylacrylamide)-chitosan (PNIPAAm-CS) polymer was formulated into a thermosensitive *in situ* gelling system for the topical delivery of timolol, an active ingredient used for the treatment of glaucoma. The PNIPAAm-CS delivery system showed a higher Cmax and area under the curve (AUC) of blood concentration against time than that of a convention eye drop containing timolol. The gel system was also able to lower the intraocular pressure (IOP) more than the eye drop over a 12-hour period (Cao et al., 2007).

Another example is a hydrogel system was developed by Yu et al containing carboxymethyl chitosan and a poloxamer composed of poly (ethylene oxide)/poly (propylene oxide)/poly (ethylene oxide) (PEO – PPO – PEO). The hydrogel was chemically crosslinked using glutaraldehyde and was able to undergo a reversible sol-gel transition in response to a change in pH and/or temperature. Preliminary studies, including cell studies performed with human cornea epithelial cells, showed that the hydrogel was not cytotoxic and has sustained drug release profiles (in comparison to a sample drug solution systems). This shows that this system could be further developed for ocular drug delivery (Yu et al., 2017).

3.6.2. Hyaluronic Acid Polymeric Bio-Platforms

Hyaluronic acid is an anionic biopolymer which is found naturally within the human body. It is biodegradable and does not cause an immune response when used in medical systems. Due to this, hyaluronic acid has been a major interest in the design of drug delivery systems. It is particularly useful in respect to ocular drug delivery because it is a component within the vitreous humor of the eye and also has ligands for receptors found in many types of retinal cells, such as CD-44 (Martens et al., 2015).

Hyaluronic acid is endogenous to the body, making it highly biocompatible and non-immunogenic. However, it is not able to form a gel on its own and thus hydrogels made from hyaluronic acid rely on chemical modifications and cross-linking or gelling agents. Hyaluronic acid hydrogels have been investigated as a drug delivery system because they are able to be formulated as both static and stimuli-response (Trombino et al., 2019).

Hydrogels are able to be utilized in conjunction with other technologies in order to improve ocular drug delivery. This can be seen in a study by Widjaja et al., where a hyaluronic acid-nanocomposite hydrogel was formulated with a sample drug, latanoprost. This system, in which the modified hyaluronic acid was combined with liposomes which contained the drug before crosslinking occurred, showed longer drug release profiles than the hydrogel and liposomes each did on their own. The composite system also improved the stability of the liposomes and the viscosity of the formulation. The hyaluronic acid was modified in two ways, using either adipic dihydrazide (ADH) or methacrylic anhydride (MA). Both modifications were tested throughout the study. The drug release mechanism is shown in the figure below; it was found that both liposomes with entrapped drug and free drug were released from the hydrogel matrix which is what is believed to be the reason behind the sustained drug delivery profile which was observed. Although only preliminary studies were conducted; with further research, these nanocomposite systems are a potential candidate for the

delivery of drugs to the eye after a single administration (Widjaja et al., 2015). Figure 5 below shows how the drug is release from the system.

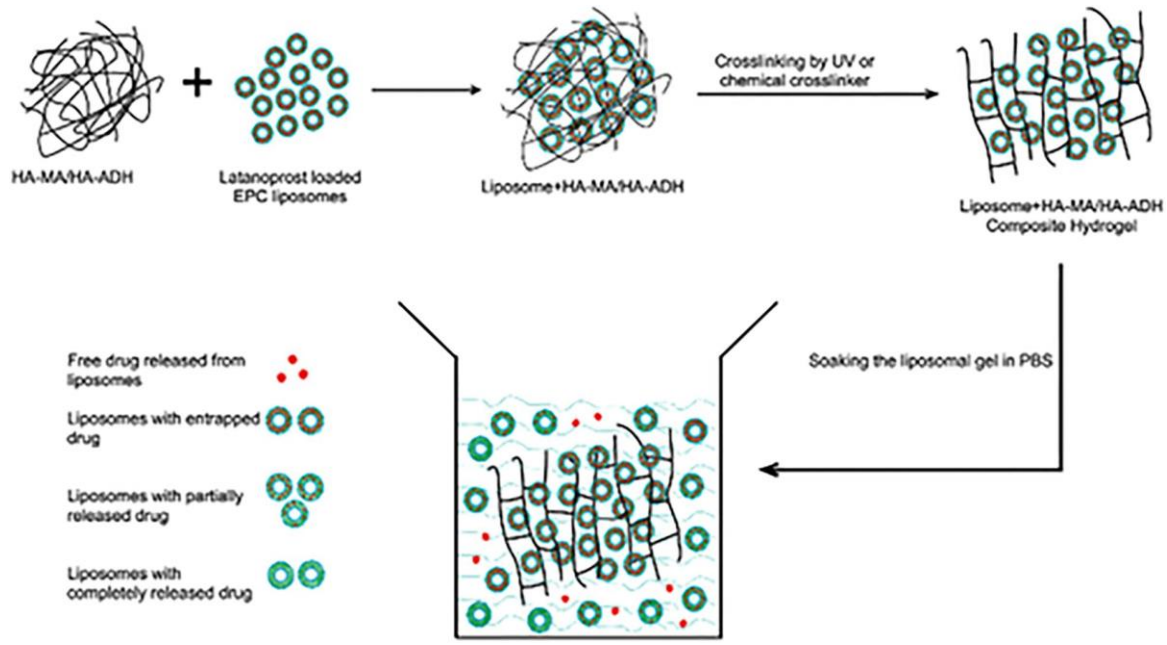


Figure 3.5. Drug release mechanism from hyaluronic acid-based nanocomposite hydrogel system. The active ingredient is loaded within the liposomes which are in turn loaded into the hydrogel. The drug is then released from the liposomes and diffuses through the hydrogel. It was also found that liposomes themselves were able to be released from the hydrogel. Both of these release mechanisms resulted in the sustained drug release seen in the formulation. This figure also highlights how the liposomes were incorporated into the hydrogel before it was cross-linked (Wadjija et al., 2015).

Another hyaluronic acid-based hydrogel system was developed by Wu et al. This system was designed to be a thermoresponsive microgel for the topical delivery of drugs to the eye. Hyaluronic acid was coupled with g-poly(N-isopropylacrylamide) to form HA-g-PNIPAAm which was shown to have high drug loading capabilities. The gel was tested for biocompatibility in rabbit eyes with the results showing that it was safe and did not cause any irritation. The formulated system, with a sample drug cyclosporine A (CyA), was tested against a castor oil solution of CyA and a commercial product also containing CyA. There was a significantly higher concentration of CyA in the corneas of rabbits who received the HA-g-PNIPAAm system than in those who received the other two solutions. This shows that in situ thernoresponsive gels are able to improve the bioavailability of ocular active ingredients (Wu et al., 2013).

Hyaluronic acid hydrogels have been investigated not only as a drug delivery system but also as an artificial vitreous substitute. Schramm et al completed a study whereby hyaluronic acid hydrogels were formulated using two different cross-linking methods: the first through the use of dihydrides as a cross-linking agent and the second through photocrosslinking. Both methods resulted in three-dimensional hydrogels which had suitable optical transparency and rubber-like consistency. The results of this study showed that these hydrogels are able to replace the conventionally used silicone oils, which have disadvantages such as the formation of cataracts and a need for surgical removal of the oil, as a vitreous replacement on a long-term basis (Schramm et al., 2012).

3.6.3. Gelatin Polymeric Bio-Platforms

Gelatin is a natural polymer which is biocompatible and biodegradable. It is derived from collagen, a substance which is found naturally within the stroma of the cornea and sclera. It has been investigated for a number of ocular drug delivery systems, including nanoparticles (Vandervoort and Ludwig, 2004). Natu et al. performed a study where gelatin hydrogels were investigated as a drug delivery system for pilocarpine, an ocular active used in the treatment of glaucoma. The hydrogels were formulated through chemical crosslinking with N-hydroxysuccinimide (NHS) and N, N-(3-dimethylaminopropyl)-N'-ethylcarbo- diimide hydrochloride (EDC). These crosslinkers were used in a variety of concentrations which altered the degree of crosslinking and subsequently the release of the drug from the hydrogel. The release of pilocarpine from the various hydrogels ranged from 29.2% to 99.2% over an eight-hour period. The hydrogels also displayed good adhesion and non-cytotoxicity profiles. This shows hydrogels comprised of gelatin to be a viable option for the delivery of drugs to the eye (Natu et al., 2007).

In a study by Song et al, chitosan and gelatin were used to form a hydrogel aimed at improving the sustained delivery of drugs to the eye. The hydrogel was formed using a double crosslinking method, using both genipin and β glycerophosphate disodium salt hydrate as crosslinking agents. The resulting hydrogel had *in situ* gelling properties; showing rapid gelation at 37°C. Timolol maleate was used a sample drug as a comparison could be made against a commercially available product. The hydrogel delivery system was non-toxic and showed a sustained release drug release profile. During *in vivo* studies, in comparison to the commercial product, the hydrogel delivery system was able to show a longer-lasting and more effective reduction (due to a 2-fold increase in duration) in the IOP. The *in situ* gelling property also prevented the system from being rapidly removed from the lower conjunctival sac by tears following administration (Song et al., 2018). Figure 6 below shows the double crosslinking-method which is used in this formulation.

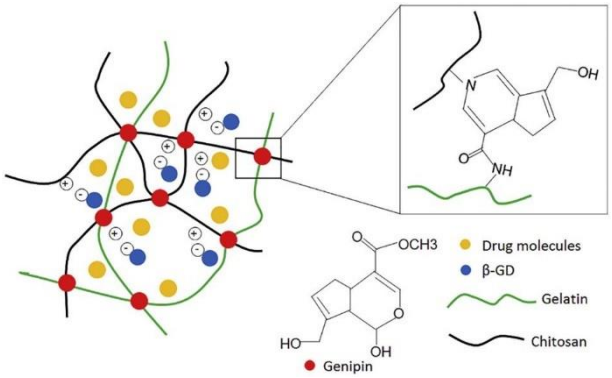


Figure 3.6. Illustration of the double crosslinking method using β glycerophosphate disodium and genipin. The β glycerophosphate disodium negatively charged phosphate groups underwent electrostatic attraction to the positively charged chitosan which gave this formulation the ability to transition between a solution and a gel (Adapted with permission from Song et al., 2018).

3.6.4. Alginate Polymeric Bio-Platforms

Alginate is another highly biocompatible polysaccharide that is able to undergo ion-responsive gelation (Liu et al., 2008). It is classified as a polyanionic copolymer and is extracted from brown sea algae. Alginate forms a hydrogel when it is exposed to divalent cations such as Ca2+ (Lin et al., 2004). It has been used in ocular hydrogel preparations because it is non-cytotoxic and biodegradable. It was used in a formulation by Lui, Z. et al which is discussed below under “ion-sensitive hydrogels”.

The utilization of alginate can also be seen in the study reported by Mandal et al where an *in situ* forming gel was prepared using sodium alginate for the sustained delivery of moxifloxacin hydrochloride, a broad-spectrum antibiotic. In this formulation, although sodium alginate was used as the primary gelling polymer, hydroxypropyl methylcellulose (HPMC) was also added as a viscosity enhancer. The resultant formulation was able to lengthen the precorneal residence time of the drug (also due to sodium alginate’s mucoadhesive properties) and improve its bioavailability. The polymer was able to undergo a sol-gel transition in response to an ion exchange when administered to the eye. *In vivo* studies were performed for biocompatibility using healthy male albino rabbits. The rabbits showed no signs of irritation after the formulation was administered to the eye and no ophthalmic damage was noted. This makes this formulation a viable alternative to conventional eye drops for the delivery of moxifloxacin with a less frequent dosage schedule (Mandal et al., 2012).

Sodium alginate hydrogels have also been used in the delivery of anti-inflammatory drugs to the eye. One such formulation is that prepared by Pandit et al. They highlighted the preference for hydrogel systems over implants as novel ocular drug delivery systems due to the fact that hydrogels are more cost effective and comfortable to the patient while still overcoming the bioavailability issues that are seen with convention drug delivery systems. The hydrogel which was produced supported these sentiments; sodium alginate was formulated into an *in situ* gelling system which would increase the residency time of the drug as well as exhibit sustained drug release profiles; both of which are vital in improving the bioavailability of ocular drugs (Pandit et al., 2007).

3.6.5. Methylcellulose Polymeric Bio-Platforms

Methylcellulose is natural polymer which is often used as a viscosity enhancer in ocular formulations. It is capable of undergoing a reversible sol-gel transition when it is heated. This makes it useful in the development of *in situ* gelling hydrogel systems (Sultana et al., 2006).

In a study Silva et al, a hydroxypropyl methylcellulose hydrogel was used to aid in the delivery of chitosan-hyaluronic acid nanoparticles to the eye, giving another example in how a hydrogel can be employed in a drug delivery system. Methylcellulose was used because it is safe to sterilize within an autoclave, it has a suitable pH for the eye and has been shown to be used successfully in other ophthalmic preparations (Silva et al., 2017). This study highlights one of the derivatives of methylcellulose, among others, which are often used in preparations. This is due to the fact that these derivatives influence the temperature at which the methylcellulose is able to undergo a sol-gel transition. For example, by lowering the molar substitution of hydroxyl propyl groups, the transition temperature is reduced from between 75°C and 90°C to 40°C (Gambhire et al., 2013).

Methylcellulose can also be added to a formulation to adjust its gelation behavior. This was investigated by Dewan et al. in a study where methylcellulose of varying molecular weights was added to Poloxamer 407 (PM), a polymer previously investigated for the delivery of various drugs to the eye. However, when used in these formulations, PM is diluted by the lacrimal fluid of the eye and loses its ability to form a gel. Increasing the concentration of PM is not a viable solution as it causes the gelation temperature to drop, resulting in the formulation turning into a gel at room temperature. It was found that the addition of methylcellulose resulted in a decrease in the gelation temperature of the PM formulations and facilitated extended drug release profiles of the sample drug; making it a viable option for sustained drug delivery to the eye (Dewan et al. 2015).

A further study which illustrates that methylcellulose can be utilized in ophthalmic drug delivery preparations is that performed by Bain et al. Agents such as fructose and sodium citrate tribasic dehydrate were added to the formulation to reduce the gelation temperature. These additives have an impact on the gelation temperature by affecting the interactions between the polymer and the water molecules. The sample drug used was ketorolac tromethamine. The resulting formulation was able to extend the release of the drug from 5 hours to 9 hours, largely due to the presence of fructose which further enhances the viscosity of the formulation. Although further testing and *in vivo* studies are needed, the resulting formulation is a viable option for the delivery of drug to the eye in the place of conventional eye drops (Bain et al., 2009).

3.6.6. Collagen Polymeric Bio-Platforms

Collagen is a natural polymer which is also available to be used in ocular drug delivery systems. Type 1 collagen is one of the primary components of the cornea and has been used in scaffolds for tissue engineering (Chen et al., 2005). Collagen shields have been formulated and are able to deliver drugs to the eye for a maximum of 72 hours. This is more beneficial than soft contact lenses, which have been shown to only delivery the drug for the first 1-2 hours after insertion. These shields are generally used following ophthalmic surgery for the delivery of anti-inflammatory or immunosuppressive active ingredients, among others. However, these shields are non-transparent and have to be applied by a surgeon (Liu et al., 2008).

However, there are some collagen shields available which have the potential to be self-administered. As reported by Khan and Khan, these bandage contact lenses are able to facilitate the healing of the cornea following surgery or injury by protecting it from abrasion caused by blinking. They are also able to be laden with active ingredients; as the tears dissolve the contact lens, the drug is released along with a layer of collagen which is able to lubricate the eye. This provides a system which is able to increase the residency time of the drug at the cornea, allowing for increased permeability and bioavailability (Khan and Khan et al., 2013).

An example of a formulation where collagen, along with hydrogel technology, has been developed is that reported by Liu et al where composite collagen hydrogels were formulated which contained alginate microspheres for the delivery of drugs to the eye. The composite hydrogels were characterized and shown to be suitable for use in ocular inserts or contact lens formulations as they were biocompatible and showed sustained drug release profiles as well as supported the attachment and growth of corneal epithelial cells (Liu et al, 2006).

Collagen has also been used in hydrogels that are intended for tissue engineering purposes. They have been investigated as an alternative to amniotic membrane which is used for clinical ocular surface reconstruction. This is due to the fact that they biodegrade at a suitable rate and offer very low immunogenicity. In a study by Mi et al., these collagen-based scaffolds were investigated. It was found that collagen gels are difficult to manipulate because of their weak structure. This was overcome through controlled unconfined plastic compression which, depending on the collage concentration and time for which the gel was compressed, produced a scaffold which closely mimicked the structure of the cornea. These hydrogel scaffolds were able to adequately support cell attachments and epithelial cell growth (Mi et al., 2010).

3.7. Safety by design of polymeric hydrogels through ocular biocompatibility and biodegradation

The eye is an organ of immune privilege, which protects its visual capability from the potentially sight-threatening sequelae of intraocular inflammation (Keino et al., 2018). Consequently, any potential formulations used in the eye, whether it be for drug delivery, tissue engineering or any other medical procedure need to be vigorously tested for biocompatibility.

3.7.1. Biocompatibility

Many studies in which new ophthalmic formulations are being investigated include biocompatibility studies. Typically, the first step in determining biocompatibility is to determine the cytocompatibility of the formulation. This is done through cytotoxicity or cell proliferation tests which are performed *in vitro*. The cell line most commonly used for these tests is human corneal epithelial cells (HCEC). These *in vitro* tests are useful in determining biocompatibility as they provide a controlled environment whereby researchers can observe the impact of the polymers used in their formulation on cell characteristics such as adhesion, proliferation and viability. It has been noted that cell studies which are performed with multiple, different cell lines provides a more accurate representation of the cells found within tissues than studies where only a single cell line is used (Huhtala et al., 2007).

The second process in determining biocompatibility is through *in vivo* testing. This is usually performed using animal models. The New Zealand white (NZW) rabbit model is most commonly used in ophthalmic bioavailability studies. This is because the eye of an adult rabbit is big enough to ensure the procedure is performed accurately (for example, rat eyes are sometimes used but are often too small for formulations designed for use in human eyes) and there is no pigment epithelium in the eye (Short et al., 2008).

Although the majority of the studies that are detailed in this review include biocompatibility studies in addition to other characterisations, either through *in vitro* or *in vivo* testing, there are those available which focus primarily on biocompatibility. One such study is that performed by Lai, J. The authors investigated,the effect of different cross-linkers (namely glutaraldehyde (GTA) and 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC)) on the ocular biocompatibility of gelatin hydrogels. Gelatin has been shown to have a rapid dissolution when it has not been cross-linked and is placed within an aqueous environment, which would limit its potential application in the delivery of drugs to the eye. The biocompatibility was tested using both cell culture techniques and *in vivo* animal testing. The cell line selected was primary rat iris pigment epithelial cells; these were cultured and observed for cell proliferation, viability and presence of pro-inflammatory genes.

The results showed that the EDC cross-linked gels were better tolerated than the GTA hydrogels. This was then corroborated in the *in vivo* tests whereby the gelatin hydrogels were inserted into the anterior chamber of the eye of NZW rabbits and observed for 12 weeks. The rabbits who were given the GTA cross-linked hydrogels showed a significant inflammation reaction whereas the EDC cross-linked hydrogels were well tolerated, concluding that EDC is more suitable as a cross-linking agent for the formulation of ophthalmic gelatin hydrogels. This study highlights that, although gelatin itself is biocompatible, the cross-linking agents which are used in the formulation of hydrogels have the ability to change the biocompatibility of a formulation (Lai, 2010).

The results mentioned in the study above were further corroborated in another study; also focusing on the biocompatibility of GTA and EDC cross-linked hydrogels, with the exception of using hyaluronic acid as the polymer. The results of the *in vivo* tests, performed using rabbits, showed that the EDC crosslinked hydrogel elicited no inflammatory response whereas the GTA cross-linked hydrogels produced a severe tissue response. This further highlights the importance of biocompatibility testing, not only for the polymer, but also for the other reactants used within a formulation (Lai et al., 2010).

Other *in vitro* methods for testing biocompatibility have been developed. An example of this is the development of a three-dimensional, curved epithelium model which is able to mimic the cornea. This model was designed and created by Postnikoff et al in the hopes of removing the need for the use of animal testing in the development of some ophthalmic preparations. This particular model was shown to be multi-layered and responsive to cytotoxic compounds, as a cornea would which makes it a viable option in the biocompatibility assessment of contact lenses (Postnikoff et al., 2014).

3.7.2. Biodegradability

Biodegradability is one of the aspects which makes the polymers discussed in this review beneficial for use in ocular drug delivery. This allows sustained drug release systems to be able to breakdown and be absorbed by the body, eradicating the necessity for surgical removal. The most common form of biodegradable system is that where a drug is embedded within a polymeric system and is released as the polymer degrades. The advantage of biodegradable over non-biodegradable ocular systems has been seen in implants developed for sustained drug release. Majority of ocular implants currently available on the market are non-biodegradable but research is being done into the development of biodegradable formulations (Lee et al., 2010).

The biodegradable nature of polymers, while advantageous, can sometimes hinder their ability to maintain their integrity for an extended time within the environment into which they are placed. For example, hyaluronic acid, which is broken down by hyaluronidase, does not have a sufficient residence time for long-term delivery. Hyaluronic acid is often modified to overcome this issue (du Toit et al., 2013).

3.8. Incorporation of Hydrogels and Nanotechnology for Ocular Drug Delivery

Hydrogels can form a vital role in the development of nanotechnologies for the delivery of drugs to the eye. An example of this is the formulation of hydrogel nanoparticles. This drug delivery system combines the benefits of a hydrogel (hydrophilic and high-water content) with the minute size of a nanoparticle. These have been developed using both synthetic and natural polymers but, in this article, only those employing natural polymers are discussed (Hamidi et al., 2008).

Although hydrogels themselves offer many advantages to overcome these challenges, by combining hydrogels in colloidal drug delivery systems the effective delivery of drugs to the eye is further improved. Nanotechnology, such as nanoparticles and nanoliposomes, has been given a lot of focus in recent years for use in ocular drug delivery. These nanocarriers are able to offer advantages such as the more targeted delivery of drugs and controlled release as well as reduced toxicity and improved efficacy of formulations. These carriers, which range from 1 to 1000 nanometers in size, are also able to deliver drugs which are poorly water soluble (a problem that in the past has seen ocular active drugs not being made into effective preparations) as well as provide improved penetration into tissues. Colloidal drug delivery systems are also able to increase the retention time at the surface of the cornea, resulting in improved bioavailability (Ameeduzzafar et al., 2016).

In terms of ocular drug delivery, nanoparticles are useful due to their small size which allows for targeted drug delivery and improved bioavailability. The drugs in these delivery systems can be incorporated into the nanoparticle either through entrapment, encapsulation or attachment to the surface. Nanoparticles with intrinsic hydrogel structure are able to be formulated using either physical or chemical cross-linking methods and have been prepared using a number of synthetic and natural polymers. Nanoparticles are able to be combined with hydrogel technology either in the way that they are synthesized or in the way that they are administered where the hydrogel acts as a suspending agent (Hamidi et al., 2008).

A further example of the combination of hydrogels and nanotechnology is nanogels. These nanoparticle carriers have many beneficial properties in terms of ocular drug delivery. These include sustained drug delivery profiles and improved stability of the drug in water (Jamard et al., 2016).

In a study by Jamard et al., it was noted that many nanogels require harsh conditions for formulation, such as high temperatures and the use of organic solvents. However, it was noted that by using biopolymers (such as methylcellulose) which have been modified with hydrophobic moieties (such as poly (N-tert-butylacrylamide)), self-assembling nanogels could be formulated through hydrophobic interaction within an aqueous environment. This renders the resultant, non-cytotoxic nanogel suitable for the delivery of biological compounds with a prolonged release profile (Jamard et al., 2016).

A further study, focusing on the delivery of fluconazole to the cornea, was performed by Nishil et al. where fluconazole loaded chitin nanogels were synthesized. The system was shown to have sustained drug release drug profiles while also being cytocompatible. It was also noted that the system allowed for penetration through the cornea in *ex vivo* studies. The nanogel can be considered for improved bioavailability for the fluconazole in the treatment of corneal fungal infections (Nishil et al., 2013).

Solid lipid nanocarriers (SLN) are another form of nanotechnology which have been researched for the replacement of conventional ocular drug delivery systems. These SLN are advantageous as they have low toxicity due to the fact that they are prepared from lipids natural to the body, are able to undergo autoclave sterilization and are able to be loaded with both hydrophilic and hydrophobic drugs (Farid et al., 2017). Solid lipid nanocarriers fall under a larger group of lipid-based nanocarriers which also includes lipid-drug conjugates (Puglia et al., 2015).

Nanoparticles offer a particular benefit in that, due to the large surface area-to-volume ratio, they are able to support a vast number of surface functional groups (Jacob et al., 2018). These surface modifications are able to improve some of the disadvantages which are seen in certain nanotechnologies. An example of this can be seen in a study by Attama et al. where a phospholipid was used as a surface modifier on SLNs. The results showed that the drug release from the SLNs which were formulated without the phospholipid happened in a burst release fashion due to the fact that there was more drug present in the periphery of the nanoparticles. In addition, a large amount of drug was found in the bulk aqueous medium. Those that were formulated with the phospholipid had a sustained drug release profile. This illustrates how surface modifications are able to have an effect on not only the drug release profiles but also the encapsulation efficacy of SLNs (Attama et al., 2008).

The concept of colloidal nanoparticulate-based systems has been investigated for therapeutic contact lenses. The incorporation of nanoparticles allows for improved drug release from the contact lens as well as prevents the interaction of the drug with the polymers of which the lens is composed. An example of such system was formulated by Jung et al. Nanoparticles which contained timolol, a drug used to treat glaucoma, were loaded onto commercial contact lenses. The contact lenses were tested in preliminary drug release and *in vivo* studies which showed that, in addition of being biocompatible, they were able to release timolol over an extended period (5 days) resulting in a lowering of the IOP. These are promising results as an alternative to conventional timolol eye drops which must be administered multiple times a day; however, there is still further research which needs to be conducted (Jung et al., 2013). This research would include the impact of colloidal systems on the contact lens' transparency and ion and oxygen permeability (Maulvi et al., 2016).

3.9. Future Perspectives

The primary focus of the research that is being done, and that has been commented on in this article is to improve the shortfalls seen in current ophthalmic treatments. Whether that be the low bioavailability and rapid clearance from the administration site found with eye drop formulations or the frequency of invasive procedures seen with intravitreal injections, future developments made in ocular drug delivery are vital (Sapino et al., 2019).

Many of the advancements being made in this area of drug delivery include harnessing the benefits highlighted for both biopolymers and hydrogel systems. One of the main focusses of the future perspectives is the further testing of the systems that have been discussed in this paper. This testing includes *in vivo* animal testing of systems that have undergone cell testing, and clinical trials for the

systems that have undergone animal pilot studies. It has been noted that not many of the newly developed systems have been made commercially available and these studies would further this process (Barbu et al., 2006).

Natural, biodegradable polymers have uses in other future prospects for ocular drug delivery outside of their use in hydrogel systems, both on their own and in conjunction with synthetic polymers. These include the development of polymeric ocular inserts (as an example, an insert was developed by Jain et al with sodium carboxymethylcellulose (CMC) and poly (vinyl alcohol) (PVA) for the topical delivery of ciprofloxacin) (Jain et al., 2010). Majority of the ocular inserts which are commercially available are composed of synthetic polymers, so the development and commercialization of biopolymer-based inserts is a definite avenue for the future prospects of biopolymer technology.

Hydrogel systems have been demonstrated in many studies to be highly beneficial in their role as ophthalmic drug delivery systems. The advances that have been made in recent years, particularly in terms of "smart" or stimuli-responsive hydrogels, have made a large impact. However, many of these formulations have not been made commercially available, mainly because many of them have yet to undergo clinical trials. This would be a vital step in improving the quality of life of patients; especially those who require eye drop administration on a daily basis. According to the research that has been done, hydrogels provide an option for far less frequent dosing schedules (in some cases weeks or months) (Chang et al., 2019).

3.10. Conclusion

Although hydrogels are not as extensively investigated as some of the other developments that are being made in ocular drug delivery, they are making an impact. These systems provide two vital benefits to drug delivery: sustained drug release and increased retention time. They are able to be formulated in such a way that they are able to respond to stimuli, which has been shown to be very beneficial. This stimuli-response ability allows for ease of administration, making these formulations more favorable for patients. This takes the ease of administration of eye drops and combines it with the increased viscosity of ointments, resulting in effective topical drug delivery without frequent dosing schedules (seen with eye drops) and blurred vision (seen with ointments).

Biopolymers are at the forefront of many studies undertaken in ocular drug delivery. These polymers, with their non-cytotoxic, biodegradable profiles enable researchers to develop technologies without the risk of causing inflammation and the need for surgical removal. They also lend themselves to

safety-by-design aspects for new formulations as there are many studies which illustrate their low toxicity profiles. Biopolymers provide an easily available and relatively cheaper option to some synthetic polymers.

Both hydrogels and biopolymers lend themselves to use in nanotechnology for ocular drug delivery. Whether it be in the form of the intrinsic make-up of the nanoparticles, nanoliposomes or nanowires, or as a suspending agent, hydrogels can greatly impact the developments which are being made in this field of drug delivery. Although there are still developments to be made, both hydrogel and biopolymer technology play a vital role in the improvements being investigated for the effective delivery of drugs to the eye.

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CHAPTER 4

FORMULATION AND CHARACTERIZATION OF A SOLID LIPID NANOPARTICLE-LOADED GEL FOR OCULAR DRUG DELIVERY

4.1. Introduction

The effective delivery of drugs to the eye remains a long-lasting challenge. This is largely due to the numerous barriers found within ocular tissues. These are present, regardless of whether the drug is being administered topically, systemically or through injection (Gaudana et al., 2010). Examples of physiological barriers in the eye include the conjunctiva and the epithelial layer of the cornea, which are known to have tight cell junctions to prevent substances and molecules passing through, the blood-aqueous barrier and the blood-retinal barrier. In addition to these physical barriers, there are physiological processes which result in the low bioavailability of ophthalmic drugs. For example, when drugs are administered to topically to the eye (particularly in the form of eye drops), they undergo rapid surface removal. This happens through the secretion of lachrymal fluid which is rapidly removed through the nasolachrymal duct as well as by the blinking reflex (Seyfoddin et al., 2010).

Glaucoma, characterized by an increase in intraocular pressure which leads to damage of the optic nerve, is one of the leading causes of irreversible blindness. The first line of treatment currently available is eye drops containing a variety of active ingredients aimed to lower the intraocular pressure. Timolol, a β-blocker, is one of the most commonly prescribed drugs which lowers intraocular pressure by inhibiting the production of aqueous humor (Xu et.al., 2019).

Eye drops, used to treat a variety of conditions which affect the anterior segment, comprise the majority of ophthalmic formulations available on the commercial market. However, they have an extremely low bioavailability, with 5% or less of the active ingredient reaching the inner tissues of the eye. Scientists have employed several different strategies to improve this, primarily investigating either an increase in the retention time of the formulation on the surface of the eye or an improvement in the penetration of the active ingredient through the various layers (Patel et al., 2013).

Nanoparticles (NP) for drug delivery, which are known to have a size ranging from 10nm to 1000nm, can improve the penetration of drugs (Zhou et al., 2013) as well as their safety. The use of lipids in the formulation of nanoparticles is particularly beneficial as they are highly biocompatible. In addition to this, lipids are able to give NP improved mucoadhesion. This allows for a longer pre-corneal retention time and thus improved permeation (Battaglia, et al., 2016). In addition to this, solid lipid nanoparticles have controlled drug release profiles (Almeida and Souto, 2007). Oleic acid (OA) was identified as the lipid for this formulation. It is known to be biocompatible within the human body and

is found naturally within the stratum corneum. In addition to this OS has been shown to increase the permeation of drugs through various physiological barriers by increasing the fluidity of the lipid bilayer. This is of particular significance as the cornea has a lipid bilayer (Gao et al., 2014). The second component of the lipid phase is Compritol®888 ATO. Compritol is a solid glycerol behenate which has been shown to have an increased loading capacity than other stearates (Seyfoddin et al., 2010).

Hydrogel formulations, which are comprised of a network of polymers, are able to improve the delivery of drugs to the surface of the eye by increasing the residency time through a higher viscosity (Cooper and Yang, 2019). In addition to this, these formulations are able to retain the therapeutic agents well and are known to be highly biocompatible (Torres-Luna et el., 2020) The polymers selected for the formulation of the gel are methylcellulose and hyaluronic acid. Both naturally occurring polymers, they are highly biocompatible with the human body. Methylcellulose is able to undergo a sol-gel transition when placed in certain conditions, one of which is a suitable temperature. This is advantageous for ocular drug delivery as the formulation can be administered accurately when in a lower viscosity while giving the formulation increased retention as a gel once in contact with the body temperature (Nagai et al., 2020). The temperature at methylcellulose undergoes the sol-gel transition can be modified through the addition of salts, polymers or through chemical or physical modifications (Das et al., 2020). For this reason, hyaluronic acid was added to the formulation. The thermosensitive nature of the gel allows for the administration of the formulation to the surface of the eye. Once the formulation has undergone the sol-gel transition, it will be retained at the administration site for a longer period of time than a solution. The drug loaded SLNs would then permeate through the gel and, due to their size, penetrate through the physiological barriers of the eye and deliver the drug to the anterior segment. This mucoadhesion property is particularly important as eye drops are known to be removed from the surface of the eye within 30 seconds from administration (Lanier et al., 2021).

This chapter focuses on the formulation of a solid lipid nanoparticle-loaded gel (SLN-G). The chemical, physical and rheological properties of the system were evaluated. The formulation was also loaded with a drug. The components of the system (SLNs and gel) as well as unloaded and drug loaded SLN-G were evaluated for their cytotoxicity. The SLN-G was evaluated for its suitability as an ocular drug delivery system through investigation of the SLN encapsulation efficiency, drug release profiles, rheological studies, thermal evaluation and biocompatibility testing, in order to provide insight into, not only the formulation of the SLN-G but also its performance as a drug delivery system.

4.2. Materials and Methods

4.2.1. Materials

Methylcellulose (MC), hyaluronic acid sodium salt (HA), timolol maleate salt (drug), PBS buffer tablets (pH = 7.2), Tween 80, ethanol and dialysis tubing were obtained from Sigma-Aldrich (St. Louis, MO, USA). Oleic acid was obtained from Merck Pty Ltd (Darmstadt, Germany). Compritol 888 ATO was obtained from Gattefosse (Saint-Priest, France). Double deionized water was obtained from a Milli-Q water purification system (Milli-Q, Millipore, Billerica, MA, USA). All other reagents used were of analytical grade and were employed as purchased.

4.2.2. Synthesis of Solid Lipid Nanoparticles (SLNs)

The SLNs were synthesized using a nanoemulsion method. The lipid phase comprised of oleic acid and Compritol 888 ATO in a 2:1 ratio was accurately weighed and heated to between 70°C and 80°C. The aqueous phase comprised of Tween 80, ethanol and ultrapure water in a 2:1:2 ratio was combined and heated. The aqueous phase was added to the lipid phase in a dropwise fashion while under magnetic stirring in order to formulate a transparent microemulsion. Following this the heated microemulsion was added dropwise to ultrapure water (ratio of 1:1) within an ice bath in order to form the solid lipid nanoparticles (Ustundag-Okur, et al., 2014). Drug loaded SLNs were synthesized by adding 5% w/w of timolol to the lipid phase before it was heated.

4.2.3. Synthesis of the Thermosensitive Gel

Methylcellulose (MC) solutions were formulated in the following concentrations: 3%, 5%, 8% and 10%. Briefly, the correct amount of MC was weighed and added to half the PBS which was heated to boiling under magnetic stirring. Once dissolved, the other half of the PBS which had been cooled in an ice bath, was added. The solutions were then placed in the fridge overnight. Hyaluronic acid (HA) was then added to each MC solution at the following concentrations: 0.5%, 1%, 1.5%, 2% and one MC concentration solution was left plain (Mayol, et al., 2014).

4.2.4. Determination of Particle Size and Surface Potential

The SLNs were analysed using dynamic light scattering and photon correlation spectroscopy at a fixed angle of 90° using a ZetaSizer NanoZS (Malvern Instruments Ltd., Worcestershire, UK) particle size analyser. The SLN solution was diluted, sonicated and filtered prior to measurement. The temperature of the samples was kept at 25° whilst under analysis. The particle size, polydispersity index and zeta potential values were measured.

4.2.5. Evaluation of Encapsulation Efficiency and Drug Loading Capacity of SLNs

The encapsulation of timolol in the SLN was evaluated in order to ascertain the drug content. Briefly, dexamethasone standards were prepared using methanol as the solvent. A linear calibration curve was constructed using a Nanophotometer UV/Vis Spectrophotometer NP80 (Implen, Munchen, Germany) at a wavelength of 295nm. The resulting formulation was centrifuged for 12 000 rpm for 30 minutes. A sample was then taken from the supernatant and the absorbance measured through UV spectrophotometry. The encapsulation efficiency was then calculated from the absorbance and based on the following equation (Equation 4.1):

$$EE = \frac{(Weight\ of\ drug\ added - Weight\ of\ drug\ in\ supernatant)}{Weight\ of\ drug\ added} x\ 100$$ *Equation 4.1.*

The drug loading capacity of the SLNs was calculated using the following equation (Equation 4.2):

$$DL = \frac{(Weight\ of\ drug\ added - Weight\ of\ drug\ in\ supernatant)}{(Weight\ of\ drug\ added - Weight\ of\ drug\ in\ supernant) + Weight\ of\ Lipid + Weight\ of\ Excipients} x\ 100$$

Equation 4.2.

4.2.6. *In vitro* Drug Release Studies

The *in vitro* release of timolol from the SLNs as well as SLN-G was tested using a dialysis tubing method adapted from Ahmed et al. (Ahmed et al., 2019). The formulation samples were transferred into the dialysis tubing and immersed in 50mL of PBS (pH 7.4) and agitated for 72 hours. Samples (1mL) were withdrawn at specified time intervals (15 minutes, 30 minutes, 45 minutes, and 1, 2, 4, 8, 12, 24, 48 and 72 hours) and replaced with an equal volume of drug-free medium in order to maintain sink conditions. The samples were then evaluated using UV spectroscopy as described in 4.2.5.

4.2.7. Chemical Evaluation of Nano-Enabled Formulation

The individual polymers and reagents used were evaluated used Fourier Transform Infra-Red (FTIR) analysis. Characteristic peaks were compared in order confirm the molecular vibrations. The FTIR spectra were obtained using a PerkinElmer spectroscope (Waltham, MA, USA) equipped with a single reflection diamond MIRTGS detector. The samples were analysed by a universal attenuated total reflectance (ATR) polarization accessory at a resolution of 4cm^{-1} and a constant pressure of 110 psi.

X-ray diffraction (XRD) was performed on the individual polymers, reagents, SLN-free gel formulation and SLN-G. This was done using a Rigaku Miniflex Benchtop Diffractometer (Rigaku Corporation, Tokyo, Japan). The crystallinity of the samples was determined using the Rigaku PDXL basis software

and the Rigaku Miniflex guidance software (version 1.2.0) guidance software used to analyse the samples.

4.2.8. Determination of Thermal Characteristics of the Nano-Enabled Formulation

The thermal properties of the individual reagents, SLNs and gel were characterized by thermogravimetric analysis (TGA) in order to determine at which temperature range the samples would degrade. This was achieved using a TG analyser (TGA 4000, PerkinElmer, Llantrisant, Wales, UK). The samples were exposed to 30° and then to 900° at a rate of 10°C/min. While under analysis, the samples were kept in an inert environment by constant nitrogen purging. The results were plotted as thermograms of percentage mass against temperature.

The phase transitions of individual reagents, nanoparticles and the gel were analysed through a differential scanning calorimeter (DSC) (STAReSystem, Mettler Toledo, Schwerzenback, ZH, Switzerland). The samples were weighed to approximately 3mg into aluminium crucibles and sealed. The crucibles were exposed to a temperature range of 0°C to 300°C at a temperature ramp of 10°C/min. Inert atmospheric conditions were maintained throughout the analysis.

4.2.9. Determination of Viscoelastic Properties of the Nano-Enabled and SLN-free Gel Formulation

The rheological properties of the gel systems (nano-enabled and SLN-free) were investigated using a Modular Advanced Rheometer System (ThermoHaake MARS Modular Advanced Rheometer, Thermo Electron, Karlsruhe, Germany) which is equipped with a C35/1° Ti sensor. Temperature ramp evaluations were carried out at a range of 20 – 40°C using a cone and plate.

4.2.10. Surface and Structural Morphological Evaluation of SLNs

The structure of the SLNs were observed using scanning electron microscopy (SEM). The samples were placed onto an aluminium stub and allowed to dry overnight in a fumehood. The images were obtained using a FEI ESEM Quanta 400F (FEI^{TM}, Hillsboro, OR, USA) electron microscope with an electron acceleration charge of 2.00 kV.

4.2.11. Determination of Cytotoxicity of SLNs and the Nano-Enabled Formulation

Caco-2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin solution. Human Retinal Pigment Epithelium cells were grown in a growth medium comprised of a 1:1 ratio of DMEM and Nutrient Mixture F-12. The cells were kept in a humidified incubator at 37°C with 5% CO_2.

In order to assess the cytotoxicity of the SLNs and the formulation 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide (XTT) assay was performed. A XTT assay uses a tetrazolium based reagent. This assay is a spectrophotometric method which indicates cell viability through the reaction of metabolically active cells with the XTT tetrazolium salt to result in the formation of formazan, an orange-coloured compound. The cells were seeded in 96-well plates at a density of $8x10^4$ cells/mL and incubated for 24 hours. Following this, the cells were treated according to the concentration of the lipids in the SLNs and the concentration of polymers in the gel and the drug-loaded formulation. The concentration of the lipids used were 135µg/mL, 67.5µg/mL, 33.75µg/mL and 16.875µg/mL. The polymer concentrations used were 2000µg/mL, 1000µg/mL, 500µg/mL and 250µg/mL. To treat the cells 150 µL of the relevant treatment was added to each well. The plates were then incubated for 48 hours and 72 hours respectively. The XTT assay was performed by combining 5mL of the XTT labelling reagent and 100 µL of the electron-coupling reagent in a reservoir. Each well was then treated with 50µL of the combined solution. The plates were incubated for 4 hours and the absorbance at 450nm and 690nm. The net absorbance value was calculated using Equation 4.3 and then used to calculate the percentage cell viability (Equation 4.4).

$$Net\ Absorbance = Absorbance_{(450nm)} - Absorbance_{(690nm)} \qquad \textit{Equation 4.3.}$$

$$Cell\ Viability\ (\%) = \frac{Net\ Absorbance_{(treated\ cells)}}{Net\ Absorbance\ _{(untreated\ cells)}} x\ 100 \qquad \textit{Equation 4.4.}$$

4.3. Results and Discussion

4.3.1 Chemical Evaluation of the Nano-Enabled Formulation

FTIR analysis of the pristine lipids, polymers and drug showed peaks which are characteristic of each component. Spectra are presented in the in Figure 4.1. The characteristic peaks of timolol were observed for the native drug such as the broad peak at 3286 cm^{-1} which indicates O-H/N-H stretching and the sharp peak at 2854 cm^{-1} which shows aliphatic C-H stretching. In addition, the carboxylic acid group and the N-H bending of the maleate salt is noted, represented by the peaks at 1702 cm^{-1} and 1489 cm^{-1}, respectively. These characteristic peaks are not seen in the drug loaded SLNs and there is no noticeable shifting of the peaks seen in the unloaded SLNs, both of which indicates that timolol is encapsulated within the SLNs rather than remaining free in the mixture, or on the surface of the SLN.

The characteristic peaks of the gel were not notably impacted by the addition of the SLN (both drug-loaded and unloaded), which shows that there is no chemical interaction occurring between the SLN and the gel components.

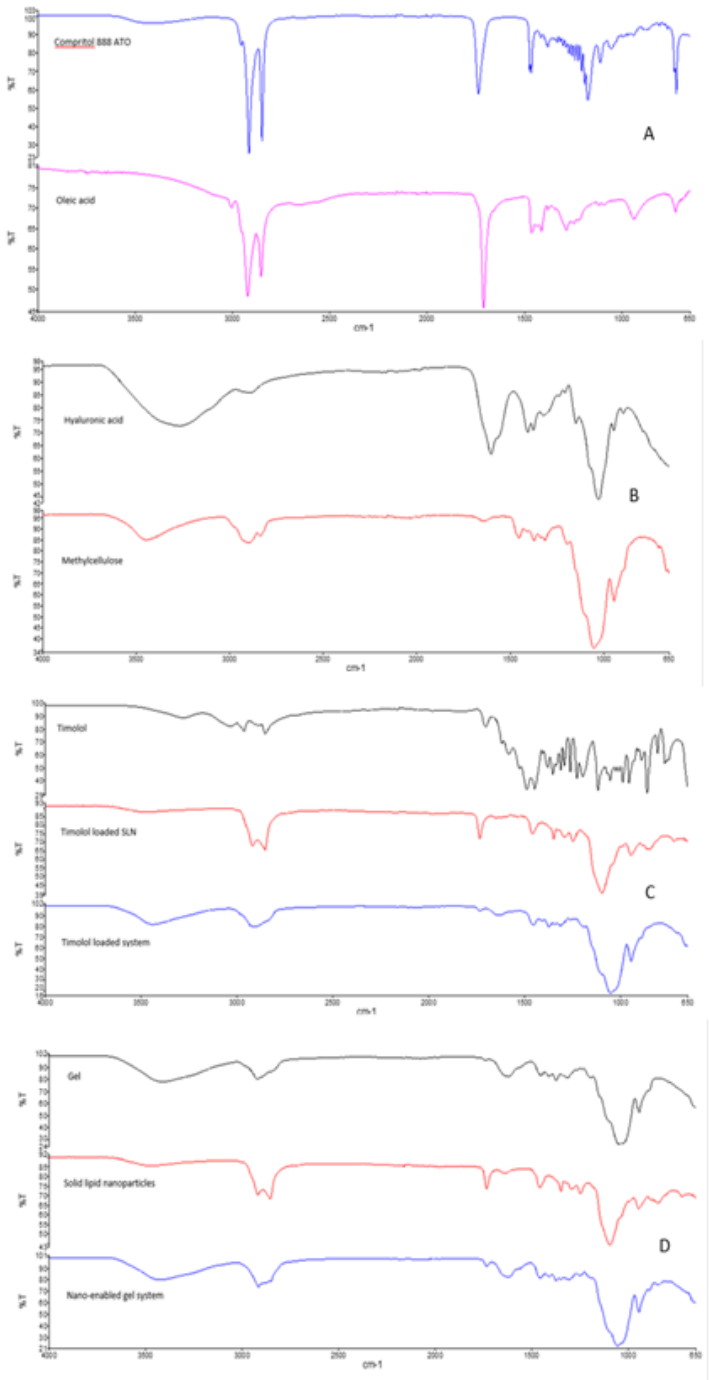


Figure 4.1. Fourier-transform infrared spectroscopy (FTIR) spectra for (A) Compritol 888 ATO and oleic acid, (B) hyaluronic acid and methylcellulose, (C) timolol, timolol loaded solid lipid nanoparticles and timolol loaded system, (D) thermosensitive gel, solid lipid nanoparticles and SLN-G.

4.3.2. Particle Size and Zeta Potential Analysis

Particle size is a vital factor in a system designed for the delivery of drugs to the eye to ensure penetration of the particle and thus the drug through the respective physiological barriers. Once the nanoparticles were synthesized, the samples were diluted and sonicated using a probe sonicator. The samples were then filtered through a 0.22 µm filter. These steps were taken in order to break up and remove any particle aggregates which had formed. The results showed an average particle size of 57.75 nm and PDI of 0.458 for the unloaded particles. The drug loaded particles showed an average size of 44.12 nm and a PDI of 0.412. By definition, nanoparticles are below 1000nm in size. However, in the case of ocular drug delivery, an average size of below 200nm is known to be highly beneficial in the penetration of the particles through the ocular barriers (Wadetwar et. al., 2020). Thus, the average size of both the unloaded and drug loaded particles would be appropriate for drug delivery. In addition to this, a PdI of less than 0.5 for both sets of nanoparticles shows that they are monodispersed.

The average zeta potential for the respective nanoparticles were -8.39 mV and -3.26 mV for the drug loaded and unloaded nanoparticles, respectively. A zeta potential of above +30 mV and below -30 mV is an indication of a stable colloidal nanodispersion (Wadetwar, et. al., 2020), while a value between the range of -11 mV and -20 mV corresponds to the agglomeration of dispersion (Lim, Kim, et.al., 2002). The addition of Tween 80 with the SLN preparation provided steric stability to the particles which would aid in creating a stable nanosuspension.

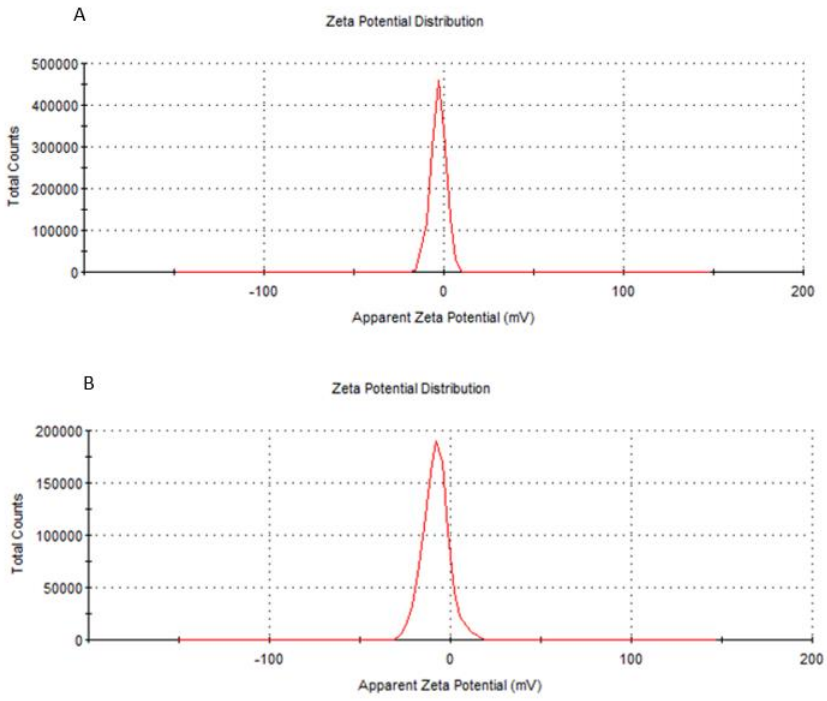


Figure 4.2. Zeta potential graphs for (A) unloaded SLNs and (B) timolol-loaded nanoenabled system.

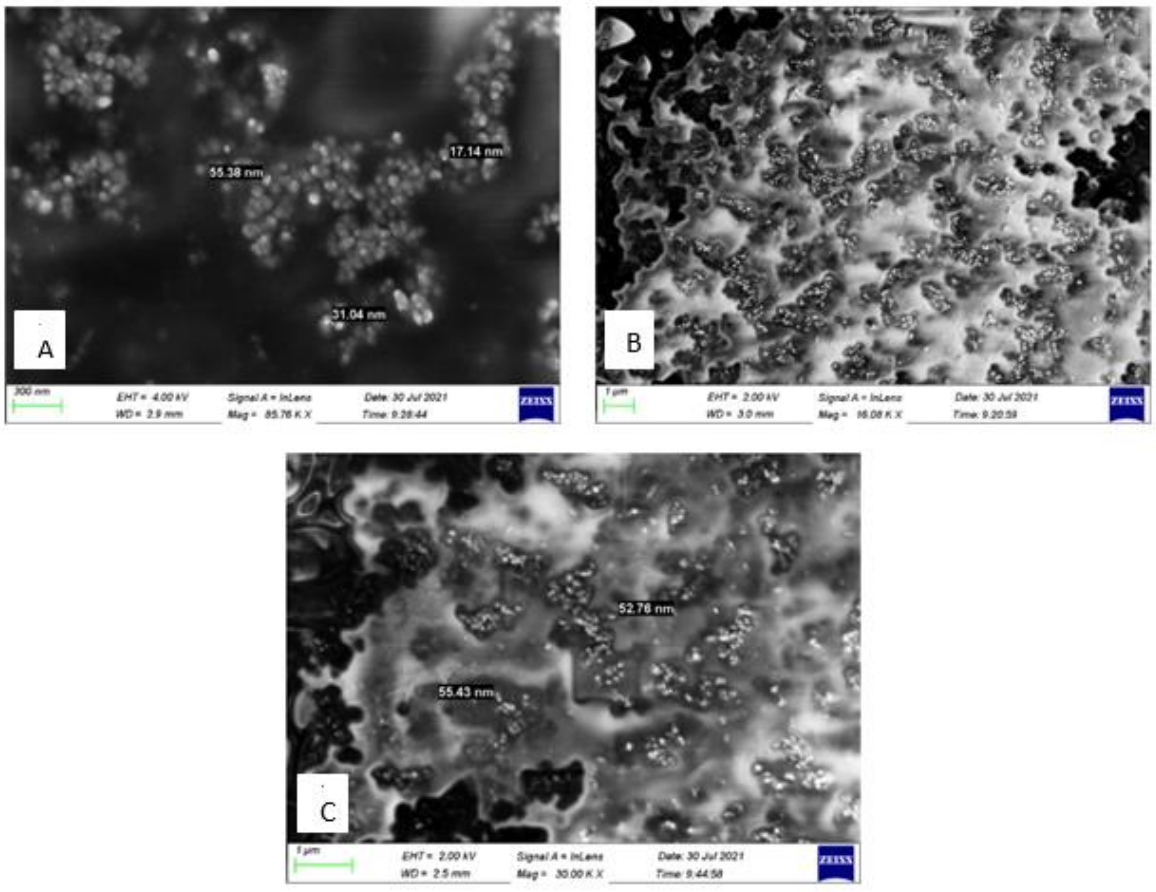
4.3.3. Drug Encapsulation Efficiency and Drug Loading of SLNs

The entrapment efficiency of the SLNs gives an indication of the percentage of drug used which is incorporated in the formulated SLNs. The drug loading capacity shows the amount of drug which is in the SLN as a comparison with the weight of the lipids and excipients used during formulation.

The SLNs showed excellent encapsulation efficiency. This can be due to the formulation procedure where the drug was completely and directly incorporated into the lipid phase of the emulsion. The drug encapsulation efficiency was calculated as 97.18% ± 0.02 and the drug loading capacity was determined to be 56.12% ± 0.05.

4.3.4. SEM Imaging of SLNs

The morphology of the SLNs were observed using SEM imaging. Due to the nature of SLN, lyophilization is ineffective prior to SEM imaging as it is known to cause aggregation and thus inaccurate imaging and sizing. The images showed the formation of spherical nanoparticles. The size of the nanoparticles measured via the ZetaSizer results were supported by the size visualised via



SEM.**Figure 4.3.** Figures A-C idicate the formulated solid lipid nanoparticles as seen through SEM magnified to 65 000 X (A), 16 000 X (B), 30 000 X (C).

4.3.5. Rheological Analysis of the Thermosensitive Gel

The effect of temperature on the gel, both unloaded and loaded with nanoparticles, under a constant applied force, was investigated. G', known to show the measure of deformation energy within a formulation, illustrates the elastic solid phase properties. In contrast to G', G'' provides a measurement of the viscous and deformation energy used and lost by a formulation over a particular temperature range under the applied force. These aspects were measured for each gel concentration combination in order to compare the changes in concentration on the gelling ability of the formulation and thus select a suitable combination. An important consideration in this aspect is the fact that a highly viscous formulation, while being effective at reducing the rapid clearance from the eye, would prohibit accurate dosing and poor patient compliance in the form of an eye drop. Thus, a concentration was selected that exhibited adequate dosing ability (lower viscosity) while also being able to from a gel at body temperature (at or below 37°C). In Figure 4.4, the rheological profile for the formulation containing 5% w/v of methycellulose and 0.5% w/v of hyaluronic acid is provided. As seen in the graph, G'' is initially higher than G'. At approximately 33°C, a complete switch occurs, which is the temperature at which gelation occurs as the formulation undergoes a significant phase transition. When the gel was loaded with SLNs, the gelation temperature decreased slightly to approximately 28°C. This is suitable as the average temperature of the human cornea is approximately 32.9°C to 36°C (Purslow and Wolffsohn, 2005).

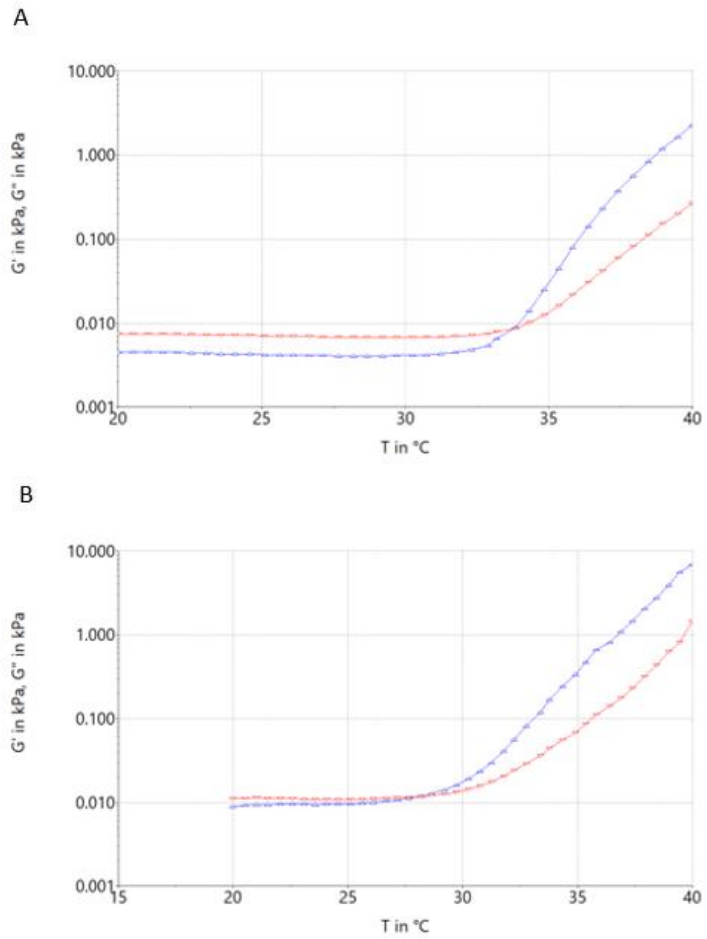


Figure 4.4. Rheometer graphs for a) the pure gel and b) SLN-G. The temperature G' and G" intersect represents the sol-gel transition.

4.3.6. Thermal Evaluation of Formulation Components, SLNs and Nano-Enabled Formulation

Two thermal evaluation techniques were performed on the reagents, gel, SLNs and SLN-G. Firstly, DSC is a technique whereby a sample is measured against a reference sample and the difference in the rate of heat flow when exposed to a controlled thermal program is analysed. This allows for the measurement of a samples melting and crystallization points. In contrast, TGA is used to analyse the thermal degradation and moisture content of the sample.

4.3.6.1. Differential Scanning Calorimetry

The DSC graphs generated (Figure 4.5) depicted the melting and recrystallization patterns of crystalline materials. In addition, information regarding the interaction between the components, drug and mixtures was evident. The melting process for Compritol 888 ATO was observed as a steep endothermic peak at 75.32 °C. This peak was not seen in the DSC graph of the SLN, showing that the lipid was in the crystalline form in the SLN. In addition to this, the endothermic peak noted at 65.32 °C on the SLN graph (higher than 40 °C) indicates that the SLN are in a solid state at room temperature

(El-Housiny, et. al., 2017). The melting point of timolol (shown in the steep peak at 201.72 °C which corresponds to the melting point reported in literature (Jagdale et al., 2016)) was not visible in the drug loaded SLN-G, indicating that the drug is incorporated in the system in the amorphous form. The addition of timolol to the system did not elicit a considerable effect, as both DSC graphs show peaks at similar temperatures. This indicates compatibility of the drug with the system.

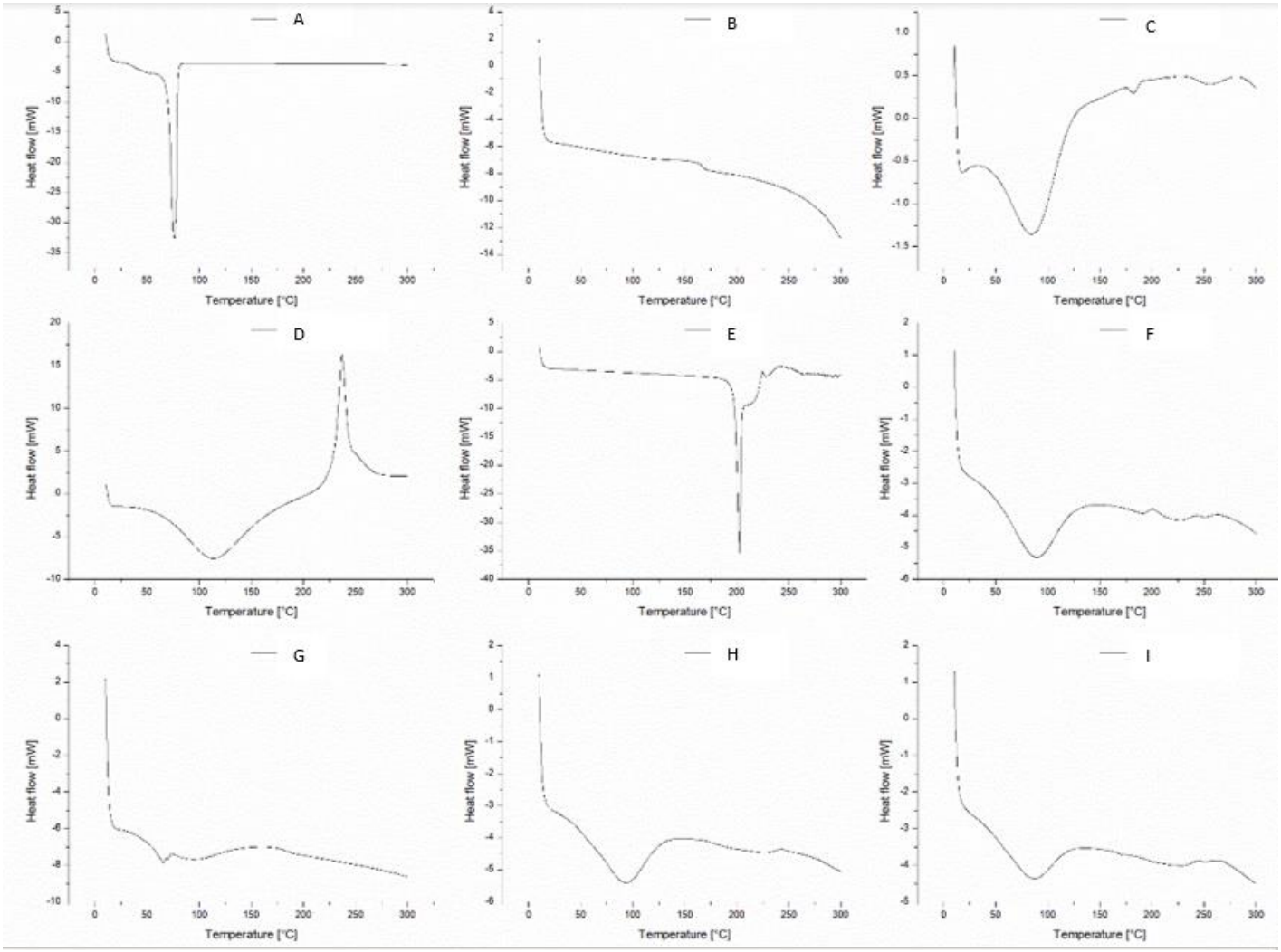


Figure 4.5. Differential scanning calorimetry (DSC) graphs for each of the pristine components as well as the formulated aspects as follows: a) Compritol 888 ATO, b) oleic acid, c) methylcellulose, d) hyaluronic acid, e) timolol, f) unloaded gel, g) SLN, h) non-drug loaded SLN-G and i) drug loaded SLN-G.

4.3.6.1. Thermogravimetric Analysis

There was no significant weight drop in the SLN sample below 180°C, indicating that the freeze-dried sample did not contain any water or ethanol, which was used during formulation (Figure 4.6). The initial weight loss seen in the hyaluronic acid sample between 41°C and 173°C can be attributed to the fact that it is highly hygroscopic. The loss of weight from 360 °C in the SLN graph indicates the degradation of the lipid components. These components, oleic acid and Compritol 888 ATO, exhibited predominant weight loss at 236°C and 289°C, respectively. This highlights that the heat range (70-80°C) used in the formulation process would not illicit any decomposition of the lipids.

There was no significant difference in the thermal properties of the unloaded system and the drug loaded system. This highlights the effective encapsulation of timolol in an amorphous state. The initial weight loss seen in both the drug loaded and unloaded SLN-G, approximately 6% in both cases, between 30°C and 90°C can be attributed to residual moisture present in the gel. The sharp weight loss then seen from 279°C and 264°C in figure 4.6 (H) and (I) respectively is the degradation of the components within the system.

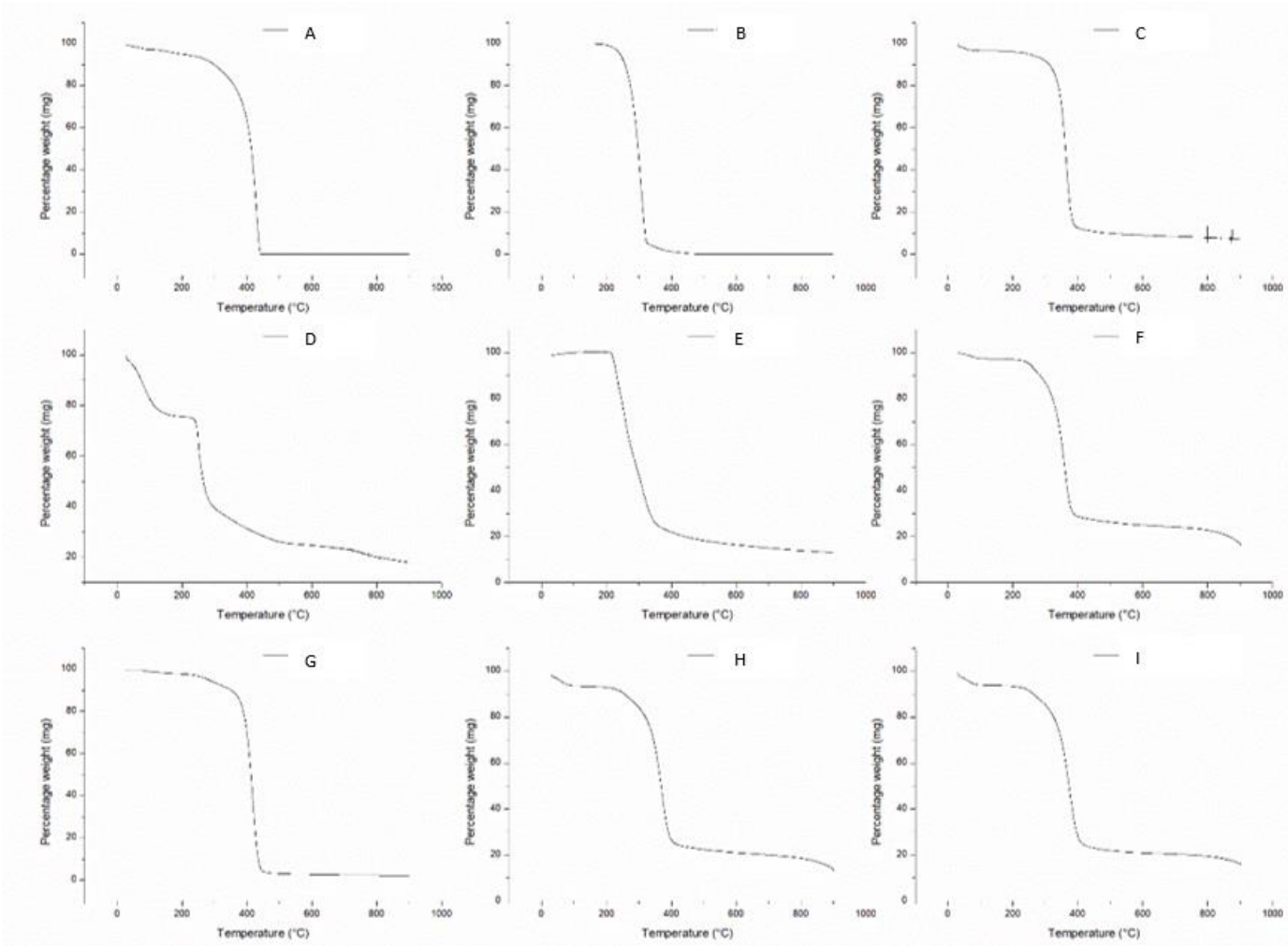


Figure 4.6. Thermogravimetric analysis (TGA) graphs for each of the pristine components as well as the formulated aspects, respectively; A) Compritol 888 ATO, B) oleic acid, C) methylcellulose, D) hyaluronic acid, E) timolol , F) unloaded gel, G) SLN, H) non-drug loaded SLN-G, I) drug loaded SLN-G.

4.3.7. X-Ray Diffraction Analysis

The prominent peak which is seen at 31.88 °C in the XRD plot of the gel (Figure 4.7), which was not seen in either component, can be attributed to the PBS which is used during formulation. This peak is typical of lyophilized PBS (Thorat, Suryanarayanan, 2019). The graphs for native hyaluronic acid and methycellulose showed the amorphous nature of the polymers and the use of PBS is believed to have led to the crystallinity of the gel. The XRD results for the gel and SLN-G showed that the prominent peaks did not shift significantly but showed decreased intensity. This highlights that the SLNs were loaded into the gel without any notable interaction. In addition, the decreased intensity of the peaks showed that the SLNs were effectively integrated into the gel (Yu, et al., 2021). The unloaded system showed distinct peaks at 27.36°, 31.76°, 45.46°, 56.54°, 66.26°, 75.42° and 84.08° at (2Θ) angle. Similar peaks were seen in the drug loaded SLN-G but with decreased intensity which indicates a decrease in the crystallinity of the system. The lack of additional peaks between the unloaded SLN-G and the drug-loaded SLN-G highlights that the drug was no longer crystalline once incorporated into the system.

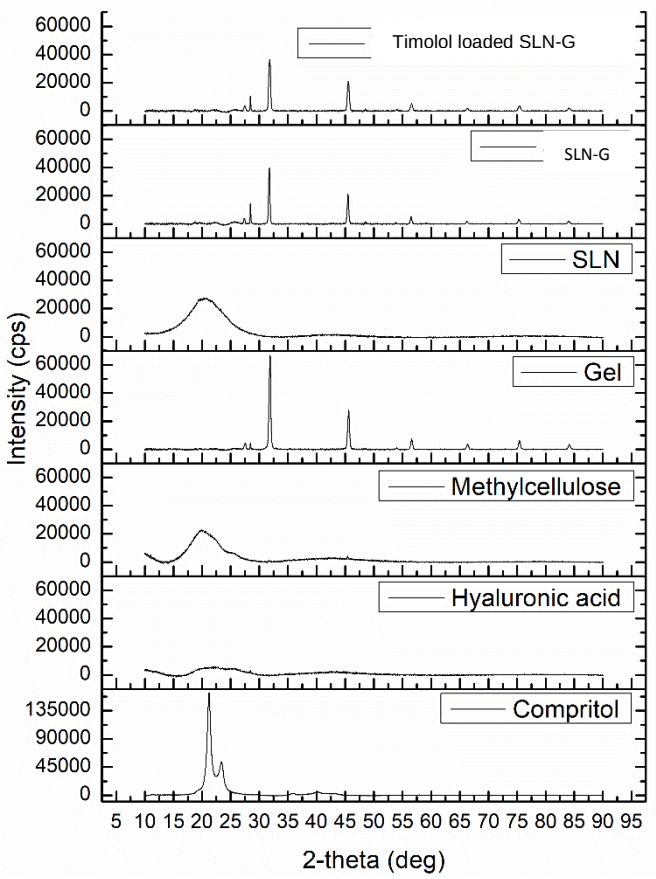


Figure 4.7. X-ray diffraction graphs (XRD) for each of the pristine components (Compritol 888 ATO, hyaluronic acid and methylcellulose), unloaded gel, SLN, non-drug loaded SLN-G and drug loaded SLN-G.

4.3.8. *In-vitro* Drug Release Study

The release of timolol from both the SLN-G and SLN was investigated. The release from the SLN-G showed an initial spike in the first 15 minutes. This can be attributed to some drug which was not loaded into the SLN but present in the solution and subsequently present as free drug in the gel, as well as drug located at the SLN surface. The decrease in release rate that followed this initial drug release could be attributed to the system undergoing the transition from a solution to a gel which would delay the release. Conversely, the free nanoparticles released the drug in a more uniform fashion throughout the 24 hours.

The SLN-G released 93% percent of the drug load within 24 hours, whereas the nanoparticles only released 66% of the drug. The improved release from the SLN-G could be attributed to the diffusion of the of the SLN through the gel. As timolol is hydrophilic in nature it would diffuse through the hydrophilic gel matrix better than through the highly lipophilic environment that would be created when the SLN breakdown on their own. Both of these profiles would be considered as prolonged release profiles, and the gel formulation could potentially be administered once daily, compared to the commercially available eye drop solutions which release the entire drug load instantly upon administration (Lanier et al., 2021), and would require more frequent application, in the case of timolol, twice a day. As the SLN-G was able to deliver close to 100% of the drug within the 24-hour period, this would allow for the delivery of the minimum effective concentration of timolol over a 24 period instead of instantaneously, as is what is seen with commercially available timolol solutions. The minimum effective concentration for the treatment of glaucoma would have been reached within the first four hours after administration. The continued release of the drug is what would allow for a less frequent dosing regimen as commercially available products need more frequent dosing in order to maintain the IOP lowering effect. The drug release profile seen in the SLN-G would illustrate the release through a Higuchi model. This model describes the release of drugs through a matrix system whereby the drug is required to dissolve out of the lipid structure and diffuse through the gel matrix before it can be released. The drug release profile observed for the SLN-G is appropriate when considering the administration site; as it would be administered to the surface of the eye, it would not be retained there for longer than 24 hours due to the physiological responses such as the blinking reflex as were mentioned earlier.

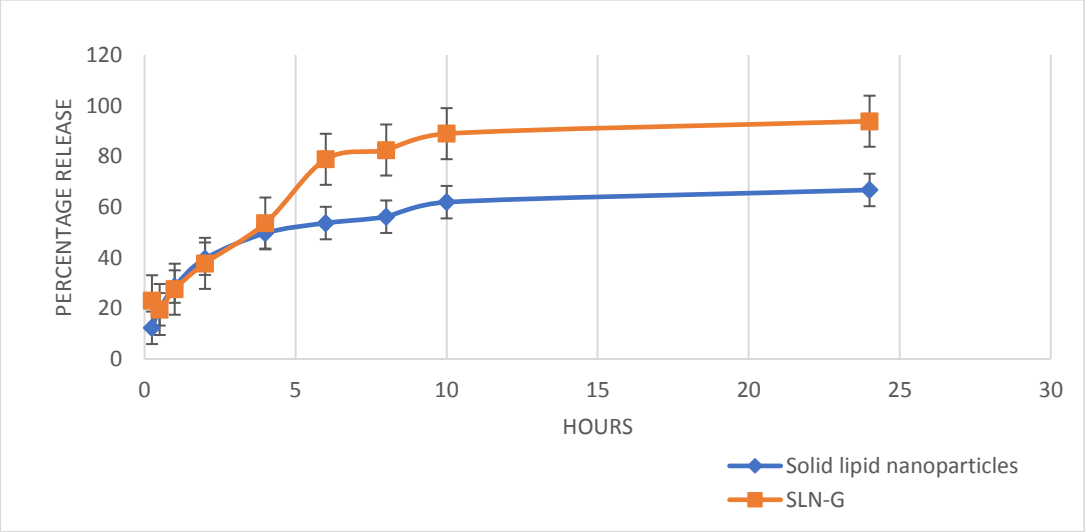


Figure 4.8. Release of timolol over a 24-hour period of both the solid lipid nanoparticles and the SLN-G.

4.3.9. *Ex vivo* Cytotoxicity Assay

Caco-2 cells were selected as they are commonly used in biopharmaceutical assessments for various reasons. They are cost-effective, fast-growing and robust while providing results which are reliable and reproducible (Kumar, et. al., 2010). All the components of the formulation were selected with their biocompatible nature in mind. Although none of the samples tested were completely cytotoxic, it was noted that the degree of cytotoxicity was dependent on the concentrations used, particularly in the case of the SLN formulations (Figure 4.9). The percentage cell viability increased three-fold when the concentration reached 16.875µg/mL in both the unloaded and drug-loaded SLNs as well as in the 48-hour samples and the 72-hour samples. The low percentage viability for the higher concentrations of SLN can be attributed to the corresponding higher level of surfactant used in the formulation process.

HRPE cells were selected as an ocular cell line. The viability of these cells was observed after a shorter period of time than the Caco-2 cells as they are faster growing and would reach confluence within the cell plate more rapidly which would negatively impact the viability of the results. The results of the HRPE mimicked those of the Caco-2 cells where the higher degrees of viability were seen in the gel and timolol-loaded SLN-G treatments (Figure 4.10). In both the HRPE cells and the Caco-2 cells, there was minimal disparity between the two time periods. This indicates that the influence of the treatments on the viability of the cells is not dependent on the time for which they are exposed.

The results of both cell lines demonstrated the biocompatibility of hyaluronic acid and methylcellulose. The viability of Caco-2 cells was 90% in the highest concentration of polymers and 92% in the HRPE cells. In addition to this, there was no correlation between the cell viability and the concentration of the polymers, as was the case in the SLN treatment.

The percentage viability of the cells which were treated with the timolol-loaded SLN-G showed that the system can be considered biocompatible. The Caco-2 cells showed a viability of over 86% for all the treatments, regardless of polymer concentration or the length of time of exposure. In the case of the HRPE cells, all the formulations showed a percentage viability of 76% and above, with an increase in viability after 48 hours of exposure. Images of both cell lines after culture and exposure to the SLN-G are shown in Figure 4.11.

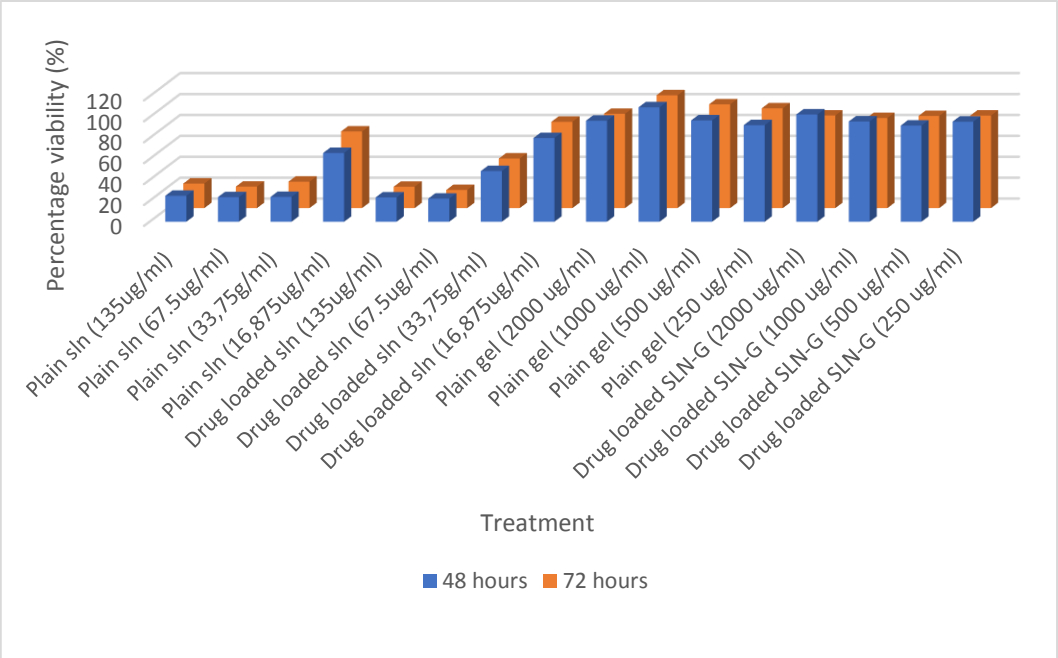


Figure 4.9. Percentage viability of Caco-2 cells 48- and 72-hours after exposure to treatment with unloaded SLNs, timolol loaded SLNs, gel and timolol loaded SLN-G.

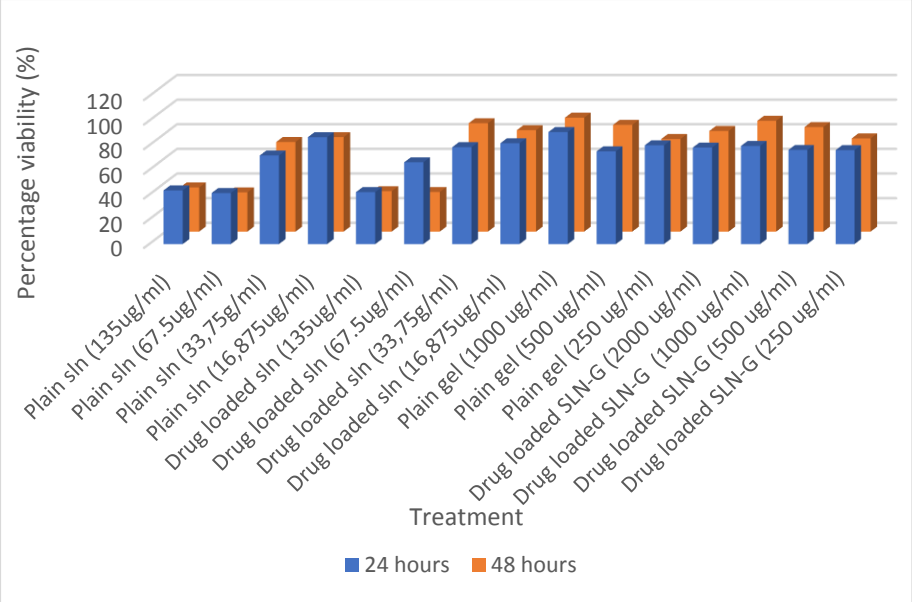


Figure 4.10. Percentage viability of HRPE cells 24- and 48-hours after exposure to treatment with unloaded SLNs, timolol loaded SLNs, gel and timolol loaded SLN-G.

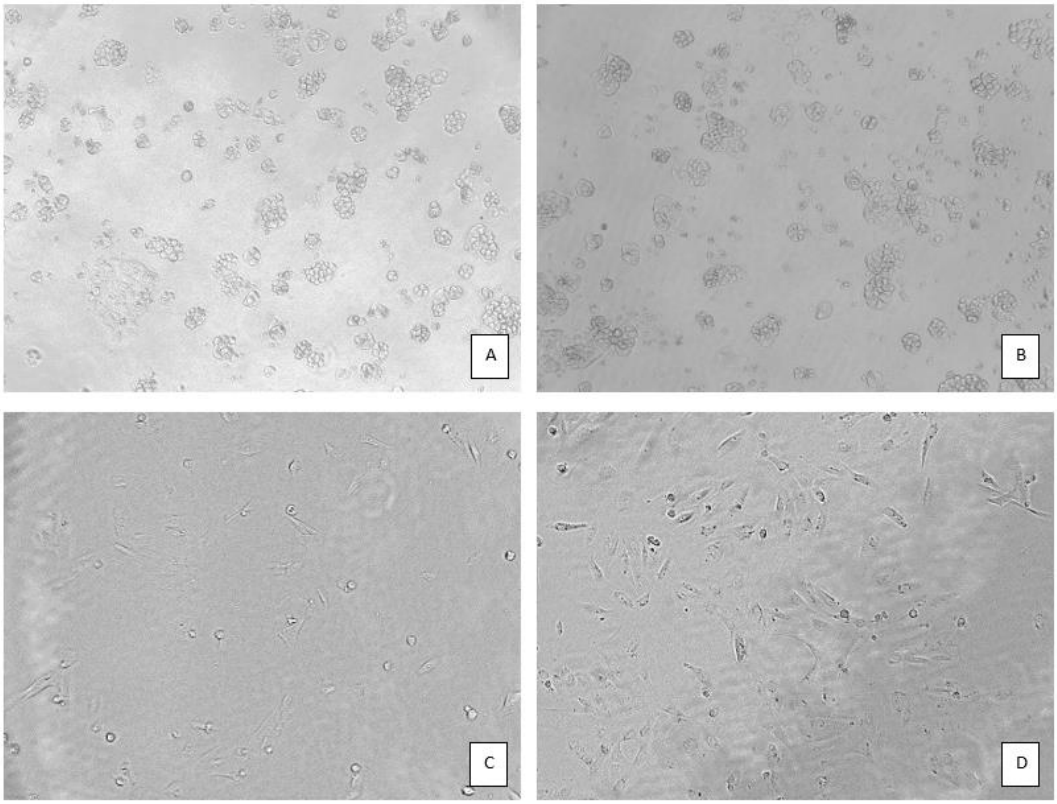


Figure 4.11. Cell culture images depicting cell viability using a light microscope at 10x magnification: (A) Caco-2 cells 48 hours after treatment with the timolol loaded SLN-G with a polymer concentration of 2000µg/mL, (B) Caco-2 cells 72 hours after treatment with the timolol loaded SLN-G with a polymer concentration of 2000 µg/mL, (C) HRPE cells 24 hours after treatment with the timolol loaded SLN-G with a polymer concentration of 1000 µg/mL, (D) HRPE cells 48 hours after treatment with the timolol loaded SLN-G with a polymer concentration of 1000 µg/mL.

4.4. Conclusion

In this chapter, a novel solid lipid nanoparticle-loaded thermosensitive gel formulation was developed. The SLNs, comprised of Compritol 888 ATO and oleic acid as the lipids, showed a suitable size and morphology for the improved delivery of drugs to the anterior segment of the eye. The gel, comprised of hyaluronic acid and methylcellulose, showed suitable thermosensitive characteristics in order to increase the residency time of the formulation at the administration site. The SLN-G possessed a favourable drug release profile and biocompatibility profile. This system could potentially perform as a viable, more efficient formulation for the delivery of drugs to the anterior segment of the eye than commercially available eye drop solutions.

4.5. References

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CHAPTER 5

CONCLUSION AND FUTURE RECOMMENDATIONS

5.1. Conclusion

This study extensively illustrates the development of an effective, innovative formulation for the delivery of drugs to the anterior segment of the eye. The literature was reviewed for the current research that is being undertaken in the field of ocular drug delivery. This included the challenges which are encountered when delivering drugs to the eye and the current treatment options that are commercially available. It was found that nanotechnology, in various different embodiments, has made an impactful inroad in this field. In addition to this, hydrogels have also been shown to greatly improve the efficiency of ocular drug delivery systems. More specifically, thermosenstive gel systems are able to exploit the efficiency of a viscous nature while enabling more accurate administration.

A thermosensitive gel, embedded with drug-loaded SLNs was successfully developed. The drug chosen for the study was timolol, a β-blocker which is used as a first line treatment for glaucoma. The formulation was developed as a more efficient alternative to timolol eye drops. As was evident in the drug release profile of the nano-enabled system, it able to deliver the drug over a 24 hour period, a notable improvement over an eye drop solution which delivers drug instantaneously.

The thermosensitive gel, comprised of hyaluronic acid and methylcellulose, was successfully developed to undergo the transition from a liquid to a more viscous gel at 33° when not nano-enabled, and at 28°C when loaded with SLNs. This temperature range is suitable for an ocular formulation which is being administered at the surface of the eye as the temperature at this site is known to be below body temperature. The thermosensitive property was incorporated into this system as it has been shown that a formulation with a higher viscosity is retained at the surface of the eye for a longer time period than those with a lower viscosity. In addition to this, the natural polymers selected for the gel are known to have mucoadhesive properties and would thus improve the residency time of the formulation at the surface of the eye. Current eye drop solutions are typically removed within a very short time period, with a large proportion of the administered dose being removed from the surface of the eye within 30 seconds. The gel was tested for cytotoxicity against both Caco-2 cells and HRPE cells and showed good biocompatibility.

Solid lipid nanoparticles, comprised of Compritol 888 ATO and oleic acid as the lipid phase, were successfully developed using a nanoemulsion technique. Through zeta sizer investigations the SLNs were found to have an average size of 57.75 nm. This was corroborated in the SEM images which

showed the presence of circular nanoparticles. This size range is highly suitable for the delivery of drugs to the eye as the small size enables the nanoparticles to pass through the tight cell junctions of the epithelial layers found in the eye. Timolol was successfully encapsulated within the SLN at an efficiency percentage of 97%.

The physicochemical properties of the drug loaded SLN-G were evaluated, including thermal degradation properties and biocompatibility. The results show favourable physicochemical properties. The SLN-G showed good biocompatibility in both cell lines in which it was evaluated, Caco-2 and HRPE.

In summary, the SLN-G which was developed can be considered to be a viable alternative to the current first line treatment – eye drops – for a number of anterior segment conditions, such as glaucoma. The goal through the development of such a system, is that the current high frequency dosage schedules of many eye drop formulations can be overcome. The gel formulation could potentially be administered once daily and maintain effective concentrations in the eye, while enhancing patient compliance.

5.2. Future Recommendations

Glaucoma can have a devastating impact on a patient's life. If not treated properly, it can lead to irreversible blindness. The effective treatment of glaucoma is the daily administration of eye drops. These solutions are known to have extremely low bioavailability, leading to frequent dosing schedules which are not always correctly adhered to by patients. Thus, an improved, more efficient formulation is greatly needed.

Much research, such as that in this study, has been undertaken in the field of ocular drug delivery. The work being evident in this field has been promising. However, it is not often that the developed formulations reach the commercial market. As mentioned in chapter two, there are a very small number of nanotechnology products available on the market. While this number has grown from only a handful of products and highlights the increase in use of nanotechnology within the medical field, it is vital that the formulations which have undergone preliminary investigations and have been shown to be effective, are studied and optimised further.

There has been a large focus in recent years on enhancing the benefits that polymer-based drug delivery systems provide. These biocompatible, and in many cases biodegradable, polymers are able to allow researchers to develop systems which are not only safe for use in the human body but are

also able to deliver drugs in a more direct manner. This allows for increased bioavailability and use of the drug as well as decreased side effects. In addition to these benefits, the ability for certain polymers to undergo changes in characteristics in response to certain stimuli provides researchers with a wide array of possibilities in terms of drug delivery systems. These *in situ* responsive polymers are able to lend themselves to systems with a number of properties, such as the release of drugs in response to a bodily trigger. *In situ* drug delivery systems are also able to enhance drug release profiles which can be extremely beneficial, particularly in the case of ocular drug delivery.

A formulation such as the one developed in this study, the results of which show favourable drug release, physicochemical and biocompatibility profiles, would need to undergo *in vivo* studies within an appropriate ocular animal model, such as the New Zealand white rabbit, in order to assure that it is effective. These studies would also highlight the permeation of the drug through the various layers of the eye. This would then lead to the development of clinical trials before the product would be made commercially available. It is vital that these avenues are pursued in order to enhance the incredible improvements both nanotechnology and polymeric science can provide are taken full advantage of.

The developed system has been shown to provide a prolonged drug release profile over 24 hours. This is extremely beneficial in the field of drug delivery to the eye as so many first line treatments, including those used in glaucoma, require daily if not multiple times a day dosing. By reducing the frequency of the dosing schedule, not only the patients' quality of life improves, but the adherence to the dosing requirements is also improved.